

# PERIODONTAL DISEASE AND THE RISK OF PRE-DIABETES AND TYPE 2 DIABETES

by  
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## Abstract

Periodontal disease is a known risk factor for diabetes in the dental literature, where most studies were cross-sectional in design and include individuals with normoglycemia and those with pre-diabetes in the same comparison groups. Despite the discussion of a bi-directional relationship for the past twenty years, evidence to support the effect of periodontal disease on the risk of incident diabetes is lacking. This dissertation explored the increased risk of insulin resistance and diabetes in response to oral inflammation. We hypothesized that oral inflammation increases the risk of insulin resistance and diabetes. This thesis consisted of three aims to test this overall hypothesis. The first aim used cross-sectional data from the 6, 138 individuals in the Atherosclerosis Risk in Communities (ARIC) Study, a community-based prospective cohort. Compared to individuals in Category I (probing depth (PD) <3mm and bleeding upon probing (BOP) ≤10%), the odds ratio for impaired fasting glucose in those with severe periodontal inflammation (Category V- one or more sites with a PD ≥4mm and BOP ≥50) was 1.5 (95%CI:1.1-2.1). A modest association between serum antibody levels to periodontal pathogens (*Porphyromonas gingivalis* and *Actinobacillus actinmycetemcommitans*) and gingival crevicular fluid levels of IL-1β and PG-E2 and pre-diabetes status was suggested but did not reach statistical significance. The second aim used the same population, where of the total 5,819 eligible participants at baseline (ARIC Visit 4), 1,967 individuals developed

incident type 2 diabetes after a mean of 13.8 years of follow-up. Incident diabetes was assessed with yearly telephone interviews and self-reports from study participants. In multivariable analyses using the Cox proportional hazards model, when compared to Category I (probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $\leq 10\%$ ), the hazard ratio of incident diabetes was the highest with early periodontal clinical measures of inflammation as found in Category II (probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $>10\%$ ) (HR=1.4, 95%CI: 1.1-1.7,  $p<0.001$ ) after adjustment for sex, age, race, education level, smoking status, physical activity, total caloric intake, waist circumference, hypertension, previous cardiovascular disease, family history of diabetes, and HDL cholesterol levels. Compared with individuals in Category I, with minimal bleeding and probing measures, the hazard of incident diabetes appears to be 1.2 times higher (95% CI: 1.0 – 1.4,  $p<0.001$ ) in adults with moderate clinical periodontal inflammation (Category IV-one or more sites with PD $\geq 4$ mm, bleeding upon probing  $>10\%$  &  $<50\%$ ) and 1.3 times higher (95% CI: 1.0- 1.6,  $P<0.001$ ) in adults with advanced clinical periodontal inflammation (Category V- one or more sites with PD $\geq 4$ mm, bleeding upon probing  $\geq 50\%$ ). The third aim was a survey of 100 Washington DC area Periodontists, to assess the attitudes and beliefs of these specialists towards the relationship of periodontal inflammation and the risk of diabetes, and how these beliefs influenced the standard of care in treating dental patients. This survey (respondents  $n=39$ ) found that practicing periodontists were aware of the

association between periodontal disease and onset of type 2 diabetes (92.9% agreed/ strongly agreed). These respondents appeared to be aware of the importance of HbA1c testing in assessing glycemic control, whether this test was performed in the dental office or medical setting. The results of this dissertation demonstrated that clinical periodontal inflammation was associated with an increased risk of pre-diabetes and subsequent incident diabetes. In addition, local periodontists understood the importance of the relationship between diabetes and periodontal disease in treating periodontal patients in clinical practice. Interventional studies are needed in the future to test whether prevention of the onset of periodontal inflammation reduces pre-diabetes and incident diabetes.

Thesis Committee- Drs. Franklin Adkinson, David Levine, Marie Diener West, and Jessica Yeh

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“The miracle isn’t that I finished. The miracle is that I had the courage to start.”

*John Bingham*

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# CHAPTER 1

## Periodontitis and Diabetes- Review of a Two-way Relationship

### Background and Rationale

Type 2 diabetes is a known risk factor for diabetes in the dental literature, where most studies were cross-sectional in design and included individuals with normoglycemia and those with pre-diabetes in the same control groups. Despite the discussion of a bi-directional relationship for the past twenty years, evidence to support the effect of periodontal disease on the risk of incident diabetes is lacking. This dissertation will explore the increased risk of insulin resistance and diabetes in response to oral inflammation. **Figure 1** illustrates the conceptual model, where exposure to periodontal inflammation, as measures by clinical measures and systemic markers specific to this periodontal disease exposure, increases the risk of insulin resistance and the subsequent onset of diabetes

**Figure 1- Conceptual Model**

## Conceptual Model

Periodontal disease increases the risk of insulin resistance and diabetes in response to oral inflammation.



### ***Type 2 Diabetes Mellitus and Established Risk Factors***

Type 2 diabetes mellitus, previously called non-insulin dependent diabetes mellitus or adult onset diabetes, is the most prevalent form of diabetes and is characterized by hyperglycemia resulting from resistance to the effects of insulin or a defect in insulin secretion. In healthy individuals, when the level of blood glucose increases, insulin is released from the pancreas to stimulate cells to remove glucose from the blood. In patients with type 2 diabetes, this high blood glucose level remains high, while these individuals are asymptomatic in early stages of disease and are often undiagnosed for several years. Symptoms of

hyperglycemia include polyuria, polydipsia, polyphagia, weight loss, fatigue and blurred vision. Increased susceptibility to infections may also be seen. <sup>1</sup>

Diabetes affects approximately 25.8 million Americans, which is over 11% of the adult population.<sup>2</sup> It has been estimated that the global burden of diabetes will increase by 54% in twenty years with a prediction of 439 million adults, or 10% of the adult population worldwide having this disease.<sup>3</sup> Risk factors for type 2 diabetes include older age, obesity, and family history of diabetes, hypertension, high cholesterol levels and history of vascular disease. Additionally women with polycystic ovary syndrome have an increased risk of diabetes. The African –American, Hispanic Americans, Native Americans, Asian Americans, and Pacific Islanders have higher risk of diabetes, compared to White/Caucasians in the United States.<sup>4</sup> Modifiable lifestyle factors include smoking cessation, increasing physical activity level, weight loss, and healthy diet.<sup>5</sup>

### ***Diabetes Complications and Burden in the United States***

The classic complications of type 2 diabetes include macrovascular disease (e.g. cardiovascular disease), microvascular disease (e.g. retinopathy, nephropathy, neuropathy), and altered wound healing. <sup>6</sup>

Cardiovascular disease appears to be more prevalent in individuals with type 2 diabetes than in those without diabetes. Compared to individuals without

diabetes, ischemic heart disease rates have been found to be about 14% higher in 18 to 44 years of age, three times higher in 45-64 years of age, and almost two times higher in 65 years of age or older.<sup>7</sup>

Diabetic retinopathy is the leading cause of blindness in adults 20 to 64 years of age with 12,000 to 24,000 new cases each year in the United States. A national population-based survey found 25% of all individuals with diabetes suffered from visual impairment, which was double the proportion of those without diabetes.<sup>8</sup>

Diabetic nephropathy accounted for over 40% of new cases of end-stage renal disease in the United States. Individuals with diabetes are the fastest growing group of recipients of dialysis and kidney transplantation in the country.<sup>9</sup>

Lower extremity disease, which includes peripheral neuropathy and peripheral arterial disease, results in increased rates of amputations in people with diabetes. Of an estimated 15 % of diabetic adults diagnosed with foot ulcers, up to 43% will progress to lower-extremity amputation due to poor wound healing.<sup>10</sup> Approximately 47% of people with diabetes had at least one lower-extremity condition (peripheral artery disease, peripheral neuropathy, insensate feet, ulcer, or lower-extremity amputation).<sup>11</sup>

Mortality among individuals with diabetes is twice that of those without diabetes and is the fifth leading cause of death.<sup>12</sup> A meta-analysis of 10 studies found that the relative risk of death was 1.85 (95% CI: 1.47-2.33) in men and 2.58 (95% CI: 2.05-3.26) in women when comparing adults with diabetes to those without diabetes.<sup>13</sup> Type 2 diabetes is now considered an epidemic in the United States and its complications account for over 130 billion dollars of health care costs in this country.<sup>14</sup> It is predicted to be one of the most common diseases in a few decades and is projected to affect at least half a billion people.<sup>3</sup>

### ***Major Clinical Trials of Prevention of Type 2 Diabetes Mellitus***

The pivotal diabetes prevention trials, including the Diabetes Prevention Program (DPP) trial, the Finnish Diabetes Prevention Program, and the China Da Qing Diabetes Prevention Study, have shaped what we know about diabetes prevention.<sup>15,16,17</sup> (**Table 1**)

The DPP trial used lifestyle interventions including weight loss, physical activity, and prescription medication in adults with pre-diabetes (impaired glucose tolerance). This study showed that intensive lifestyle intervention reduced the development of diabetes by 58%. This study also found that lifestyle changes were more effective than the use of metformin (31%) in reducing diabetes onset when compared to placebo.<sup>15</sup>

The Finnish Diabetes Prevention Study (DPS) used intensive lifestyle intervention in its intervention group involving individualized nutritional counseling

from a nutritionist, circuit type resistance training, and advice to increase overall activity during the first year, followed by a maintenance period. This resulted in 3.5 kg weight reductions over 3 years. During the first 3 years of the study, 22 adults (9%) in the intervention group and 51 (20%) in the control group developed diabetes ( $P= 0.0001$ ).<sup>16</sup>

China's Da Qing Diabetes Prevention Outcome Study randomly assigned 542 patients with impaired glucose tolerance into one of three intervention groups (diet, exercise or diet plus exercise groups) or a control group consisting of only a physical exam. With 6 years of lifestyle intervention, they found a 47% reduction in the incidence of severe retinopathy over 20 years due to the reduced incidence of diabetes (77.4% developed diabetes at follow-up in the treatment group vs. 90.3% developed diabetes in the control group).<sup>17</sup>

However, a meta-analysis of randomized educational and behavioral interventions (ranging from 1-19 months) in individuals already having type 2 diabetes found only modest improvements (0.43%) in glycemic control (with follow-ups ranging from 1-16 months) with these approaches.<sup>5</sup> This study recommended more research be conducted to define the interventions needed to produce consistent improvements in glucose control after the onset of diabetes.

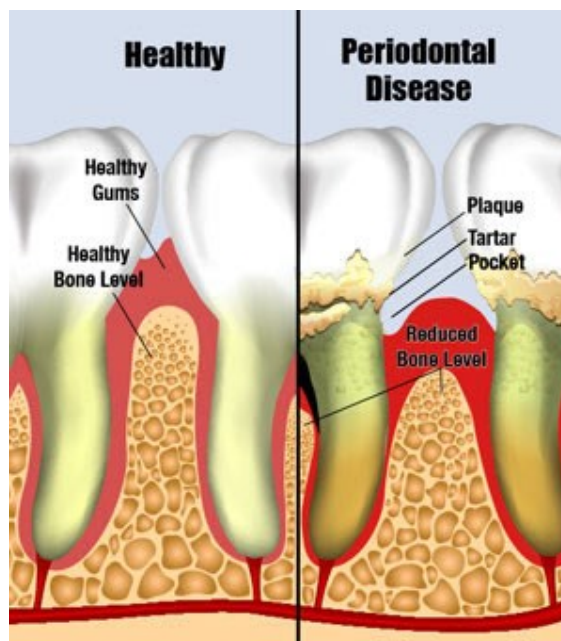
**Table 1- Pivotal Randomized Clinical Trials for Diabetes Prevention with Lifestyle Interventions**

Study	Design N	Interventions	Control	Comments
DPP <sup>15</sup> 2009 United States	N=2776	Lifestyle Medication	Placebo	10 yr follow-up  Reduction in diabetes by 34% (24-42%) in intervention vs. control 18% (7-28%)
DPS <sup>16</sup> 2003 Finland	N=522	Intensive diet and exercise for first year	General diet and exercise advice	3 yr follow-up  Weight reduction (3.5 kg) in intervention vs. control (0.9kg) with improved glycemic and lipids measures
Da Qing <sup>17</sup> 2011 China	N=542	Diet Exercise Diet and Exercise	Physical Exams	6 year follow-up  47% reduction in severe retinopathy in combined intervention group attributed to reduced incidence of diabetes.

### ***Periodontal disease definition***

Periodontal disease is defined as loss of attachment of the periodontium, whereby gingival epithelial cells and connective tissue attachment, and bone around the tooth migrate apically (downwards) away from the cemento-enamel junction. This loss of periodontal tissue is caused by the host response to mostly gram-negative bacteria and their toxins found in plaque. It is quite common in the U.S. adult population and is often seen clinically and radiographically after the age of 35 years old, with moderate periodontitis affecting 40-60% of adults and advanced periodontitis affecting 10-15% of the U.S population. <sup>19</sup>

**Figure 2: Periodontium: Healthy vs. Disease<sup>63</sup>**





## ***Type 2 Diabetes and Clinical Periodontal Disease***

There is a clear relationship between the degree of hyperglycemia and gingival inflammation.<sup>18</sup> In addition, type 2 diabetes is a known risk factor for perioontontitis in the dental literature.<sup>19</sup>

Four studies were identified that evaluated the longitudinal glycemic control in patients and their association with periodontal health.<sup>20 21 22 23</sup> These studies all controlled for age and smoking, but the other confounders varied considerably. All studies used partial mouth periodontal exams and the outcomes assessed for glycemic control varied for each study. These studies may not be generalizable to the general population in the United States. (**Table 2**)

A meta-analysis of 10 interventional studies of periodontal treatment found that successful periodontal therapy did not result in statistically significant changes in glycemic control in diabetic subjects, with 0.57% reduction in A1c measures ( $p=0.82$ ).<sup>24</sup> However, most of the studies were small; only 456 subjects were included in all ten studies. Larger studies with randomized clinical trials are needed to determine the benefit of periodontal therapy on glycemic control in patients with diabetes.

**Table 2 -Effect of periodontal disease on glycemic control on adults with type 2 diabetes or without diabetes.**

Author Year Country	Design  N	Diagnosis	Outcome	Effect Size	Confounders	Results
Taylor et al. <sup>20</sup> 1996 USA	Cohort-5yrs Pima Indians with diabetes  105	Probing  Use of dental X- ray exams	OGGT A1c	Severe vs. no perio dz.  OR=6.2 (1.5- 25.3)	Age Smoking	Periodontitis is associated with poor glycemic control in diabetic individuals after 2-5 years.
Morita et al. <sup>21</sup> 2010 Japan	Cohort-4 yrs No diabetes  1,023	Probing	OGGT	>= 1 MetS vs. no MetS  OR=1,6 (1.1-2.2)	Age Gender Smoking Exercise Diet Weight	Periodontitis is associated with metabolic syndrome (MetS) in healthy subjects after 4 years
Demmer et al. <sup>22</sup> 2010 Germany	Cohort-5 yrs No diabetes  2,793	Probing # of teeth	A1c change from baseline	Those with no perio dz had 0.005% ↑ in A1c than those with perio dz Which had 0.143% ↑ in A1c, (p=0.003) over 5 yrs	Age Waist: hip ratio BP Triglycerides Physical activity WBC Fibrinogen CRP Sex Region Smoking Education	Periodontal disease is assoc. with ↑ A1c levels
Saito et al. <sup>23</sup> 2004 Japan	Retrospective- 10 yrs No diabetes 961	Probing	OGGT A1c	High vs. low probe depth groups OR=2.4 (1.4-2.6, P= 0.009) for risk of IGT	Age Sex Smoking BMI Exercise Alcohol	Probing depth was associated with glucose intolerance

## ***Periodontal Disease and Systemic Inflammation***

Acute endotoxemia, by injection of *E. coli* lipopolysaccharide (LPS) has been shown to induce insulin resistance in cell receptors in adipose cells.<sup>25</sup> In periodontal disease, LPS endotoxin is expressed on cell walls of periodontal pathogens such as *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*. These endotoxins act via TLR 4 to trigger inflammation and loss of periodontal attachment around teeth. While both pathogens may be present in active periodontitis, *Porphyromonas gingivalis* is commonly associated with a chronic slowly progressive generalized form of periodontal disease and *Actinobacillus actinomycetemcomitans* is more commonly associated with an aggressive form of periodontitis, which can present clinically in younger ages. Antibodies are produced to these periodontal pathogens. These serum antibody titers are the most specific markers to reflect systemic exposure to periodontal pathogens. Inflammatory mediators, such as Prostaglandin E<sub>2</sub>, have also been measured in gingival crevicular fluid (GCF) collected from the gingival crevice to assess periodontal disease.<sup>26</sup>

Both diabetes and periodontal disease have been found to result in an elevation of inflammatory cytokines as a host response. Gram-negative bacteria found in periodontal disease have been found to result in elevated levels of these cytokines, such as Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in both the gingival crevicular fluid and in peripheral blood in diabetic patients with periodontal disease. Individuals

with diabetes and advanced periodontal disease had two-fold higher levels of PGE<sub>2</sub> and Interleukin-1 $\beta$  (IL-1 $\beta$ ) when compared to individuals with diabetes and milder forms of periodontal disease.<sup>27</sup> Similarly, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), another cytokine commonly associated with periodontitis, was found to exacerbate insulin resistance.<sup>28</sup> Salvi et al. however, found only marginal elevations of TNF- $\alpha$  in diabetic individuals with periodontal disease when compared to non-diabetic individuals with periodontal disease. Interleukin-1 $\beta$  (IL-1 $\beta$ ) is expressed in both patients with periodontal disease and diabetes and is believed to play a role in the pathogenesis in both diseases.<sup>29</sup> Kurtis et al. showed that gingival crevicular levels of IL-1 $\beta$  were highest in individuals with diabetes (2.43  $\pm$  0.97 ng/ml), followed by those with periodontitis (1.31  $\pm$  0.92 ng/ml) and these elevations were significantly higher than those in the healthy controls (0.62  $\pm$  0.58 ng/ml,  $p < 0.05$ ).<sup>30</sup> Protein kinase C, produced by neutrophils in response to periodontal disease, was found to be highly correlated with glycosylated hemoglobin levels ( $r = 0.71$   $p < 0.001$ ).<sup>31</sup> Thus, the hypothesis of a bidirectional relationship between periodontal disease and diabetes may be due to the inflammatory response to periodontal disease as measured by specific serum markers as well as clinical measures.

### ***Systemic inflammatory Markers and Type 2 Diabetes***

Markers for inflammation, such as high white blood cell count, predict the onset of incident diabetes with an odds ratio of 1.9 (95% CI: 1.6- 2.3) in a 7-year longitudinal study of the ARIC cohort including 1, 457 participants without

diabetes at baseline.<sup>32</sup> C-reactive protein (CRP), an acute phase response protein, was elevated in a cross-sectional study of subjects with diabetes.<sup>33</sup> However, CRP has been found to be elevated for reasons other than diabetes, such as advanced periodontal disease, obesity, stroke, myocardial infarcts or other infections and is not specific to exposure to diabetes. Serum interleukin-1 beta (IL-1 $\beta$ ), another measure of systemic inflammation, has been found to be elevated in 50 patients with diabetes and gingival inflammation when compared to 30 patients with diabetes but healthy gingiva in a cross-sectional study (2.9 +/- 3.2 pg/ml vs. 1.5 +/- 1.4 pg/ml; p=0.008.)<sup>34</sup>

### ***Insulin Resistance at the Cellular Level***

Insulin resistance at the cellular level may be a mediator of inflammation and type 2 diabetes. Inflammatory cytokines are known to activate cell signaling phosphorylation cascades such as MAP-kinase and NF $\kappa$ B pathways.<sup>35</sup> These pathways have multiple effects on cellular activities to include insulin resistance, insulin secretion and further cytokine production. (**Figure 3**), and the resulting associated oxidative stress has been found to be a significant negative modifier to antibodies to oral pathogens.<sup>36</sup> An animal model inducing periodontal disease in lean rats (n=24) found an elevation of fasting glucose (p=0.003), insulin, (p=0.008) and insulin resistance (p<0.001) as evaluated through paired analysis with Zucker fatty littermates (n=24).<sup>37</sup> The Zucker fatty rat is a known model of prediabetes, with hyperinsulinemia, dyslipidemia, and moderate hypertension.

This animal study has been the first to look at the progression to a pre-diabetic state that can be attributed to the induction of periodontal inflammation as an independent risk factor. Periodontitis was found to affect glucose tolerance in lean rats when compared to lean rats without periodontal inflammation.

### ***Effect of Periodontal Treatment on Type 2 Diabetes***

Identifying modifiable sources of inflammation might lead to novel approaches to prevent type 2 diabetes. Studies aimed at assessing the effect of treatment of periodontal disease on metabolic control of diabetes have yielded conflicting results. One study found a 10% reduction in glycosylated hemoglobin values with non-surgical periodontal and antibiotic therapies in 113 diabetic subjects ( $p=0.04$ )<sup>38</sup> In this study, 5 subgroups of diabetic participants all received periodontal scaling and either chlorhexidine oral rinse, low dose systemic tetracycline, chlorhexidine rinse and doxycycline, povidone-iodine rinse and doxycycline, or placebo (saline rinse). At 3 months post-treatment, the doxycycline treated groups showed the greatest reduction in periodontal inflammation with decreases in probing depths and detection of *Porphyromonas gingivalis*. Other studies however have looked at similar outcomes after periodontal treatment in a meta-analysis finding that the overall reduction in glycosylated hemoglobin (A1c) in subjects with diabetes mellitus after non-surgical periodontal therapy was 0.57% for four studies. This reduction was not statistically significant ( $p=0.82$ ).<sup>24</sup> These intervention studies all used clinical assessment to determine successful periodontal therapy in persons with diabetes

compared to those with normoglycemia . The non-significant effect of periodontal therapy on glycosylated hemoglobin does not imply that periodontal therapy has no effect on this pathway completely, since glycosylated hemoglobin is not sensitive to immediate or short-term effects on insulin resistance, and four of the studies ranged from only 2 to 8 weeks duration.

Thiazolidinone, an anti-diabetic medication used to improve insulin sensitivity, has been shown to inhibit LPS *Porphyromonas gingivalis* induced cytokine production in adipocytes in vitro.<sup>39</sup>*Porphyromonas gingivalis* is not completely eradicated even after successful periodontal therapy. It is biologically plausible that the most sensitive assessment of exposure to periodontal inflammation involves periodontal pathogens and measures of their systemic levels, such as *Porphyromonas gingivalis* serum antibodies.

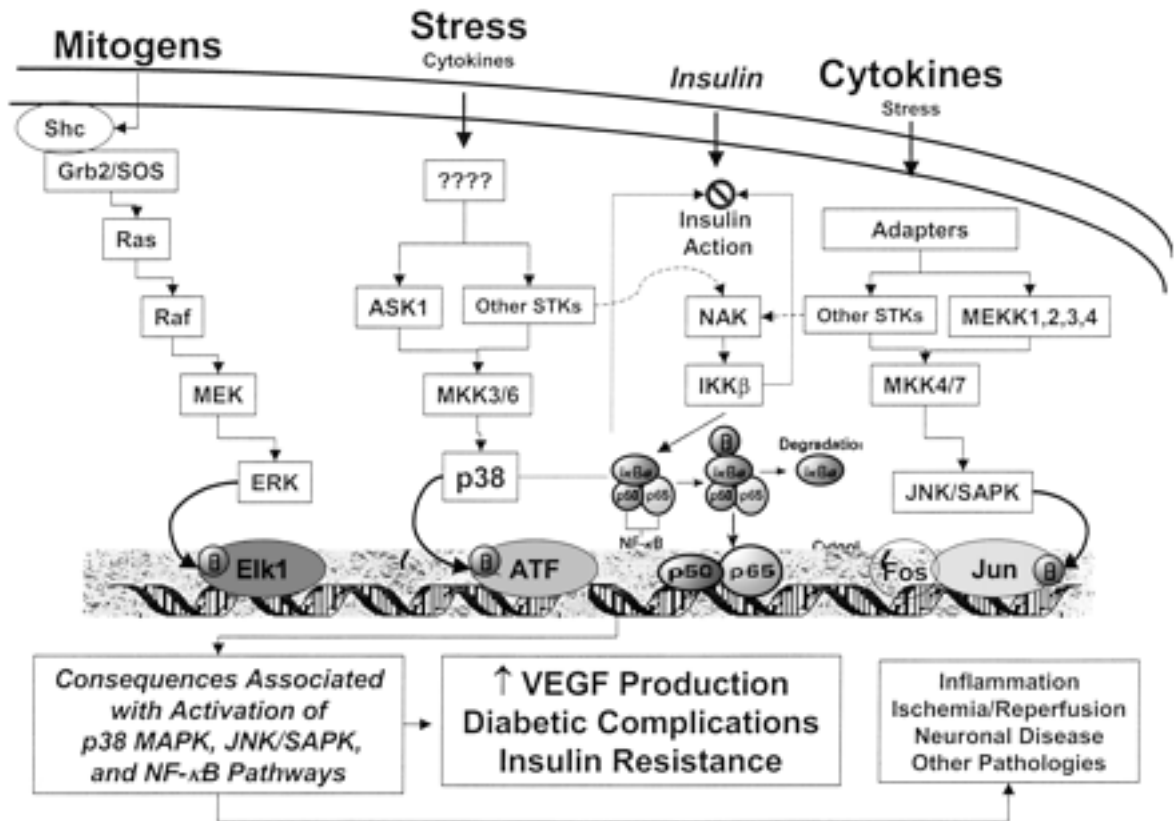
### ***The Directionality of Periodontal Disease and Diabetes***

Periodontal disease has been proposed as one source of inflammation that might predispose adults to developing diabetes. Though the hypothesis of a bidirectional pathway between periodontal disease and diabetes has been proposed, few studies have addressed periodontal disease before the occurrence of diabetes.<sup>19</sup> Periodontal disease has also been shown to increase the risk of other systemic conditions such as cardiovascular disease in adults and poor pregnancy outcomes.<sup>40 41</sup> Localized periodontal inflammation is now known to have systemic effects on general health. Compromised oral health may

increase the risk of a pre-diabetic status mediated through diet and inflammation. However, there are no known published longitudinal clinical studies of exposure to periodontitis, which use both clinical exams and systemic markers for inflammation, and the subsequent risk of diabetes.



**Figure 3: Relationship of Inflammatory Cytokines with Induction of Insulin Resistance**<sup>34</sup>



## ***Main Hypothesis/Study Questions***

**Our central hypothesis is that periodontal disease leads to systemic inflammation and thereby to insulin resistance and future type 2 diabetes.**

To test our hypothesis, we conducted two related analyses—one cross-sectional, one longitudinal, using data from community-based cohort study, ARIC Study. Finally, a survey to assess the attitudes and beliefs of Periodontists about the association of periodontitis with type 2 diabetes was conducted.

### ***Specific Aim 1***

#### **Hypothesis:**

Periodontal disease, characterized by evidence of periodontal disease on clinical examination, high serum IgG titers to oral pathogens, and localized oral markers in gingival crevicular fluid are cross-sectionally associated with impaired glucose tolerance (IGT), and elevated fasting glucose (FG).

### ***Specific Aim 2***

#### **Hypothesis:**

Exposure to periodontal inflammation, (using clinical exam evidence, systemic inflammatory markers and local inflammatory markers), predicts the subsequent occurrence of incident type 2 diabetes.

### ***Specific Aim 3***

#### **Hypothesis:**

The association of periodontitis with diabetes with type 2 diabetes is accepted by local Peridontists in the Washington DC area , and the attitudes and belief of these specialists influences the standard of care in treating dental patients.

## Chapter 2

# The cross-sectional association of periodontal disease and pre-diabetes and undiagnosed diabetes

### ***Abstract***

Periodontal disease is the most common inflammatory condition worldwide and diabetes is quickly becoming a global epidemic. The bidirectional pathway of periodontal disease and diabetes is not fully understood. While consistent evidence has shown that diabetes is related to periodontitis, emerging evidence suggests that periodontal disease may increase the risk of diabetes onset. Using data from the Atherosclerosis Risk in Communities (ARIC) Study, a community-based prospective cohort, the associations of clinical measures, local inflammatory markers, and systemic markers specific to periodontal inflammation with pre-diabetes were assessed. Compared to individuals in Category I (probing depth (PD) <3mm and bleeding upon probing (BOP)  $\leq 10\%$ ), the odds ratio for impaired fasting glucose in those with severe periodontal inflammation (Category V- one or more sites with a PD  $\geq 4$ mm and BOP  $\geq 50$ ) was 1.5 (95%CI:1.1-2.1). A positive association between serum antibody levels to periodontal pathogens (*Porphyromonas gingivalis* and *Actinobacillus actinomycescomitans*) and

gingival crevicular fluid levels of IL-1 $\beta$  and PG-E2 and pre-diabetes status was suggested but did not reach statistical significance, indicating the associations between local and systemic markers for periodontal inflammation and pre-diabetes was modest.

## ***Introduction***

An extensive body of literature consistently identifies the association of type 2 diabetes with periodontal disease.<sup>42</sup> Impaired glucose tolerance, or pre-diabetes is a requisite for type 2 diabetes onset. Most published studies have focused on the effect of diabetes on periodontal inflammation.<sup>20-23</sup> However, most models proposed to explain the relationship between diabetes and periodontal disease have focused on a 2-way, bi-directional interaction between these two diseases. Certain inflammatory mediators, such as IL-1 $\beta$  and PG-E2, have been associated with both diabetes and periodontal disease. (**Table 3**). These inflammatory mediators are inducers of acute phase proteins such as CRP, and these mediators have been shown elsewhere to impair intracellular insulin signaling. Previous studies that reported the relationship of inflammatory mediators common to both periodontal disease and diabetes were small cross-sectional studies, and included subjects with impaired glucose tolerance in the healthy patient category.

This study analyzes the cross-sectional association of periodontal disease, (characterized by evidence of periodontal disease on clinical examination, high serum IgG titers to oral pathogens, and localized oral markers in gingival crevicular fluid) with impaired glucose tolerance (IGT), and elevated fasting glucose (FG) from the ARIC (Atherosclerosis Risk in Communities) Study. This population is a biracial, ongoing prospective, community based study designed to assess clinical and subclinical atherosclerosis in adults aged 45-64 years of age. While the initial intent of the ARIC cohort design was to study cardiovascular disease, this population provides a rich database to assess the cross-sectional association of periodontal disease with pre-diabetes. This is the first study to assess the association of clinical measures, local inflammatory markers, and systemic markers specific to periodontal inflammation with pre-diabetes.

Our study uniquely looks at a large population of pre-diabetic individuals and their; clinical parameters of periodontal inflammation (bleeding upon probing, and probing depths); systemic markers of exposure to periodontal inflammation (serum IgG levels of antibodies to pathogens *Porphyromonas gingivalis* and *Actinobacillus actinmycetemcomitans*); and local inflammatory markers of periodontal inflammation (gingival crevicular levels of IL-1 $\beta$ ). The ARIC database provided a rich access to clinical, localized, and systemic markers specific to periodontal inflammation with which to assess the association of periodontal disease with pre-diabetes.

**Table 3- Studies of the association of markers of periodontal inflammation with diabetes.**

Study	Design N	Inflammatory marker	Laboratory assay technique	Comments
Engbretson et al. <sup>43</sup>  2004	Cross- sectional  45 adults with type 2 diabetes and chronic periodontitis	IL-1 $\beta$ in gingival crevicular fluid	ELISA	IL-1 $\beta$ in GCF correlates with glycemic control in diabetes independent of clinical periodontitis  Patients with greater than 8% HbA1c had significantly higher mean GCF IL-1beta levels than patients with less than 8% HbA1c.
Kardesler et al. <sup>44</sup>  2008	Cross- sectional  17 adults with diabetes and chronic periodontitis, 17 with chronic periodontitis only, and 17 healthy controls	IL-1 $\beta$ and PG-E2 in gingival crevicular fluid	ELISA	IL-1 $\beta$ was lower in diabetes patients with chronic periodontitis as compared to patients with chronic periodontitis and no diabetes (p<0.001)
Andriankaja et al. <sup>26</sup>  2009	Cross- sectional  340 adults with healthy gingival (30 of whom had type 2 diabetes)	IL-1 $\beta$ and PG-E2 gingival crevicular levels	ELISA	Gingival crevicular levels of IL-1 $\beta$ and PG-E2 levels elevated in gingivitis, irrespective of diabetic status, serum IL-1 $\beta$ levels elevated in gingivitis subjects with

	385 adults with gingivitis (50 of whom had type 2 diabetes)			diabetes to those with gingivitis and no diabetes (2.9 +/- 3.2 pg/ml versus 1.5 +/- 1.4 pg/ml; P= 0.008).
Ebersole et al. <sup>45</sup>  2008	Cross-sectional  39 Hispanic Americans with type 2 diabetes  24 non-diabetic controls	Serum antibody levels of <i>P. gingivalis</i> , <i>A.actinonycetemcomitans</i> , and <i>Campylobacter</i>	DNA checkerboard hybridization of plaque and ELISA analyses of serum IgG	Antibodies to periodontal pathogens were found more frequently in subjects with diabetes compared to those without diabetes (p<0.05)

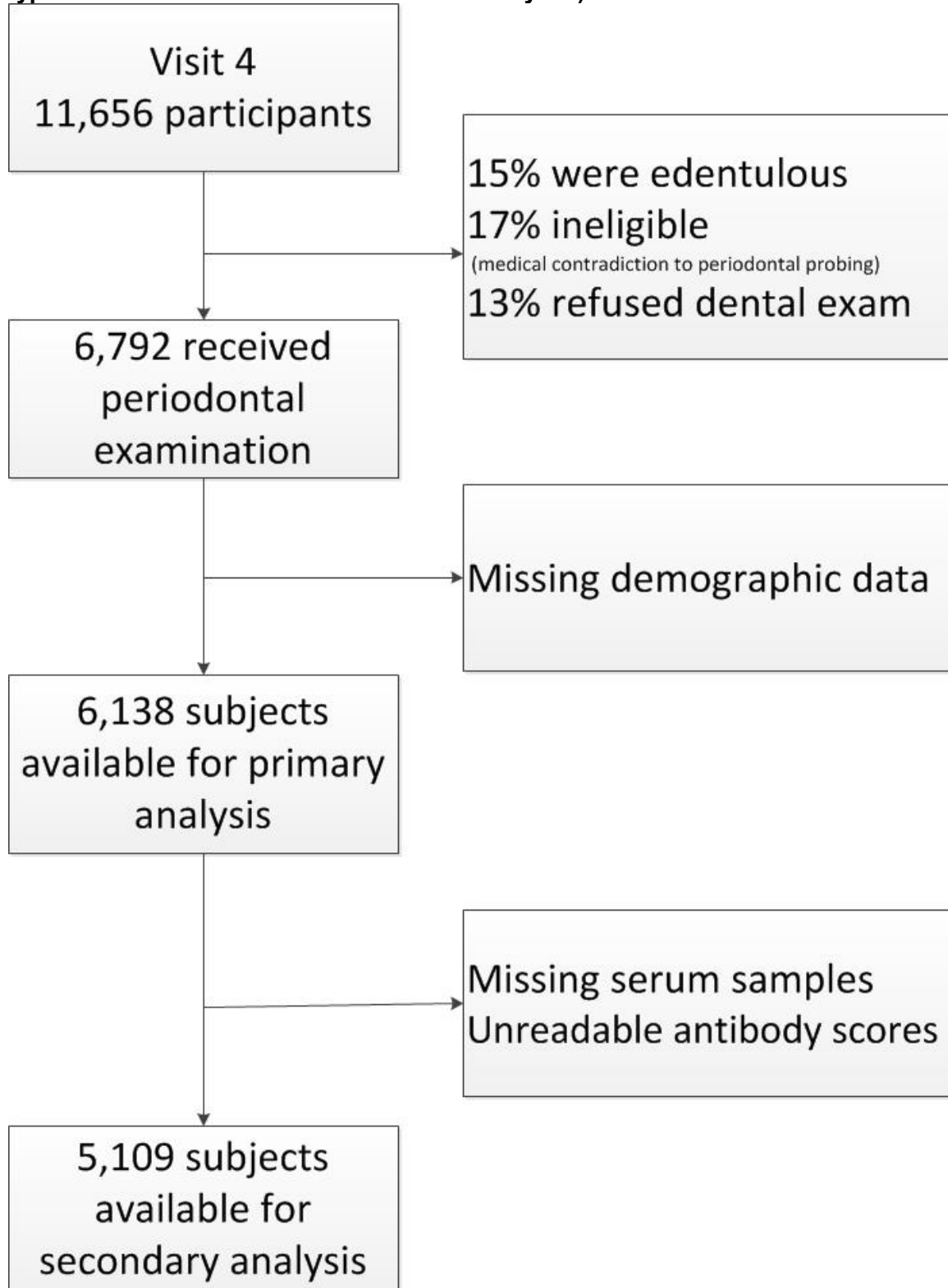


## Research Design and Methods:

### *Description of Cohort*

The Atherosclerosis Risk in Communities (ARIC) Study is a community-based prospective cohort of 15,792 middle-aged adults from four U.S. communities. The first examination of participants (visit 1) took place during 1987–1989, with three follow-up visits taking place: each approximately every 3 years. The Dental ARIC study, an ancillary study, funded by the National Institute of Dental and Craniofacial Research (NIDCR), was conducted during ARIC visit 4 in 1996 through 1998 and was cross-sectional in design. The Dental ARIC consisted of an oral examination, collection of serum, and interviews. Of those 15,792 ARIC cohort members examined at baseline (1987 to 1989), responders to a screening interview were selected. Respondents with no teeth or a medical contraindication to probing were excluded, while some refused the dental exam. A total of 11,656 ARIC participants were seen at visit 4 and 6,792 underwent the periodontal examination. After excluding adults with type 2 diabetes ( $n=421$ ) or missing demographic data ( $n=133$ ), the number with dental examinations decreased to 6,138. In the analysis of serum markers, additional exclusions were applied when serum samples were not available or antibody level were not readable ( $n=1,029$ ). Therefore, 5,109 adults were included in the analysis of inflammatory marker levels. Missing serum samples further reduced the number antibody level assessments to 5,109. (**Figure 4**)

**Figure 4- Participants in the cross-sectional analysis (doctor diagnosed type 2 diabetics excluded from 1° and 2° analyses)**



## ***Periodontal Disease***

Clinical assessments of periodontal inflammation were defined by two assessments: bleeding upon probing and periodontal pockets (rounded down to the nearest mm). This definition is consistent with the standard of care in assessing the clinical periodontal status. (**Appendix- Figure 17**) Using these two parameters, participants were classified into 5 categories: <sup>46</sup>

- I) probing depth (PD)  $\leq 3\text{mm}$ , bleeding upon probing  $\leq 10\%$
- II) probing depth (PD)  $\leq 3\text{mm}$ , bleeding upon probing  $> 10\%$
- III) one or more sites with  $\text{PD} \geq 4\text{mm}$ , bleeding upon probing  $\leq 10\%$
- IV) one or more sites with  $\text{PD} \geq 4\text{mm}$ , bleeding upon probing  $> 10\%$  &  $< 50\%$
- V) one or more sites with  $\text{PD} \geq 4\text{mm}$ , bleeding upon probing  $\geq 50\%$

Serum markers of prior periodontal disease exposure was defined by 1) serum IgG antibodies to the periodontal pathogens *Porphyromonas gingivalis* and 2) serum IgG antibodies to *Actinobacillus actinmycetemcommitans*. These variables were measured as the level of antibody response to the periodontal pathogen *Porphyromonas gingivalis* and *Actinobacillus actinmycetemcommitans* in Elisa units (EU). Using the upper quartile as the cut-point, the high antibody group was compared to the low antibody group (lower three quartiles).

*Porphyromonas gingivalis* antibody levels were considered high at  $\geq 78.93$  EU,

and *Actinobacillus actinmycetemcomitans* antibody levels were considered high at  $\geq 144$  EU. The use of the upper quartile for assigning the high antibody level group has been used on other studies.<sup>51</sup> The normal antibody level in periodontal health for these periodontal pathogens has not yet been established.

Local inflammatory markers of periodontal disease were assessed using gingival crevicular fluid levels of IL-1 $\beta$  (GCF- IL-1 $\beta$ ) and gingival crevicular fluid levels of prostaglandin (PG-E2). The variable GCF- IL-1 $\beta$  was measured as the level of gingival crevicular fluid units (ng/mL). Using the upper quartile as the cut-point, participants were considered to have high levels of GCF- IL-1 $\beta$  levels at  $\geq 146$  ng/mL. The variable PG-E2 was also measured as the level of gingival crevicular fluid units (ng/mL) and a dichotomous variable (high/low) was used. Subjects were considered to have elevated levels of PG-E2 levels at  $\geq 239$  ng/mL using the upper quartile cut-point. Similarly to the antibody levels to *P.g* and *A.a*, normal levels of IL-1 $\beta$  and PG-E2 in periodontal health have not been established.

All clinical periodontal measures, as well as serum and gingival crevicular samples were measured at visit 4 (1996 through 1998).

### ***Diabetic Status Categorization***

Individuals with type 2 diabetes were excluded from this analysis. The ARIC visit 4 individuals were classified as having a diabetes diagnosis if any of the following criteria were met; self-report of current use of medication for diabetes or blood sugar; or a positive response to the question “Has a doctor ever told you that you had diabetes (sugar in the blood)?”. Undiagnosed diabetic individuals were classified as having fasting glucose of at least 7.0mmol/L (126mg/dL); non-fasting glucose of at least 11.1mmol/L (200mg/dL), but no doctor diagnosis of diabetes and no self-report of anti-diabetic medication. These ARIC definitions at the time of visit 4 were based on the 1997 American Diabetes Association criteria.

Participants were asked to fast for 12 hours before the ARIC visit 4 clinic visits and to bring all current medications to determine medication use. Glucose was measured using the hexokinase method, and individuals were classified as having normoglycemia, impaired glucose tolerance, or undiagnosed diabetes, using the 2013 American Diabetes Association criteria<sup>49</sup> (**Appendix Figure 19**): normal glucose (fasting glucose <100 mg/dL and 2 hour glucose tolerance test <140 mg/dL, and no diabetes diagnosis); impaired glucose tolerance (2 hour glucose tolerance of 140-199 mg/dL and no diabetes diagnosis); impaired fasting glucose (FG from 100-125mg/dL, and 2 hour glucose<140 mg/dL and no diabetes diagnosis: or undiagnosed diabetes (FG >125 mg/dL, or 2 hour glucose

>199 mg/dL and never been told by doctor that one has diabetes/or no current use of diabetes medication.

### ***Other Variables***

Covariates measured at the visit 4 baseline included sex, age, race, education, smoking, physical activity, total caloric intake, BMI, waist circumference, hypertension, previous cardiovascular disease, family history of diabetes, and high density lipoprotein levels. Information on age, sex, race, smoking, total caloric intake, education level and family history of diabetes were based on self –report. BMI (weight in kilograms divided by the square height in meters) and waist to hip ratio (in centimeters) were measured with standard procedures.<sup>47</sup> Prevalent cardiovascular disease was based on self-report, ARIC clinical exam, or hospital records. The physical activity was assessed using a modified version of the questionnaire developed by Baecke and colleagues, from which a sport index was derived, ranging from 1 (lowest) to 5 (highest).<sup>48</sup> HDL cholesterol levels were measured after dextran-magnesium precipitation. The education levels, however were measured earlier, at visit 1 (1978-1989), and then were dichotomized into  $\leq 12$  years or  $>12$  years of education.

### ***Data Analysis***

Baseline differences between characteristics of normoglycemic individuals (fasting glucose $<100$ mg/dL & 2hr glucose tolerance test $<140$ mg/dL, and no

diabetes), impaired glucose tolerance (2hr glucose tolerance test=140-199mg/dL, and no diabetes), impaired fasting glucose (FG=100-125mg/dL, and 2hr glucose tolerance test<140mg/dL and no diabetes), and individuals with undiagnosed diabetes (FG>125mg/dL or 2hr GTT>199mg/dL & no diabetes diagnosis) were compared for visit 4 using ANOVA tests for continuous variables and  $\chi^2$  for categorical variables (**Table 4**). Means and frequencies of each potential confounder were also determined for each categories of clinical periodontal inflammation (Category I-V- **Table 5**). ANOVA and  $\chi^2$  analyses were used to assess the statistical differences across the 5 categories.

Multiple logistic regression models were fitted to describe the cross-sectional association between clinical periodontal disease and pre-diabetic status after adjustment for potential confounding variables. Additional multivariable analyses were performed to investigate the roles of inflammatory markers (antibody levels to periodontal pathogens *Porphyromonas gingivalis* and *Actinobacillus actinmycetemcomitans* and serum gingival crevicular levels of IL-1 $\beta$  and PG-E2) as potential exposure variables. Adjustment for confounding factors in these models included sex, age, race, education level, smoking status, physical activity, total caloric intake, BMI, waist circumference, hypertension, previous cardiovascular disease, family history of diabetes, and HDL cholesterol levels. Since waist circumference was highly correlated with BMI (corr=0.89), only waist circumference was chosen to remain in the final models. Current

smoking status was used in the models due the expected effect on clinical periodontal measures.

In all multivariable models, tests for interactions with pre-diabetic status were performed with sex, race, waist circumference and smoking status. No interactions were detected (all  $P > 0.05$ ), and therefore only pooled results were presented. All tests of significance were two-tailed, with a  $\alpha$  level of 0.05. All analyses were performed using STATA 9.1 (Stata, College Station, TX).

## Results

Baseline characteristics by pre-diabetic status were presented in **Table 4**. All the characteristics were considered statistically different between the groups if  $p < 0.05$ . Compared with individuals with normal glycemia, in this cohort, adults with pre-diabetes and undiagnosed diabetes are more likely to be female, African American, older, and less educated. Furthermore, individuals with pre-diabetes had a higher BMI, waist circumference, history of hypertension, and family history of diabetes ( $P < 0.01$ ). There was no difference in total caloric intake ( $P = 0.1253$ ) or previous history of cardiovascular disease by category of pre-diabetic status ( $P = 0.3020$ ). A subsidiary analysis of baseline characteristics of 4,864 individuals available at visit 4, but excluded from the periodontal examination, was performed (results not shown). The individuals excluded from our primary analysis had a higher proportion of African-Americans (39% vs. 24%), smokers



(19% vs. 13%), increased caloric intake (1773 kcal/day vs. 1587kcal/day) and higher body mass-indices (31kg/m<sup>2</sup> vs. 28.9kg/m<sup>2</sup>).

Baseline characteristics by clinical periodontal inflammation status (category I-V) were presented in **Table 5**. Individuals in the categories with more severe clinical inflammation were more likely to be female, smokers, and less physically active. These periodontal groups also displayed higher caloric intake, BMI, waist circumference, hypertension, previous cardiovascular disease, HDL cholesterol, fasting glucose and 2 hr glucose tolerance levels. (P<0.01) There were no differences in age (P=0.34) and family history of diabetes (P=0.11).

The distribution of clinical category by glycemic status in 6, 138 ARIC Dental Study participants is displayed in **Table 6**. High proportions of study participants (between 29.0% in individuals with undiagnosed diabetes, and 42.9% in individuals with normoglycemia, P<0.0001) were displayed with moderate periodontitis (Category IV periodontal status- one or more sites with PD >4mm, bleeding upon probing >10% &<50%). Likewise, the distribution of inflammatory markers in normoglycemia, IGT, IFG, and undiagnosed diabetes in 5,109 ARIC Dental Study participants without diagnosed diabetes is displayed in **Table 7**, with all inflammatory markers showing no statistically significant associations with glycemic status (all P-values were >0.05).

In the analysis of 2 hr GTT (**Table 8**), severe clinical periodontal inflammation (Category V) was associated with elevated risk of impaired glucose tolerance in an unadjusted model with an odds ratio of 1.3 (95% CI: 1.0-1.7). However after adjustment for lifestyle and co-morbidity variables, this association attenuated to null. (OR=1.0, 95% CI: 0.7-1.3).

As Shown in **Table 9**, compared to individuals in Category I, participants with more severe periodontal clinical inflammation had increased odds of impaired fasting glucose. Compared to individuals in Category I, the odds ratio for impaired fasting glucose in Category V was 2.1 (95% CI: 1.6-2.8) in an unadjusted model. This relationship remained in the fully adjusted model with an odds ratio of 1.5 (95% CI: 1.1--2.1) in the highest category of one or more sites with a probing depth >4 mm and bleeding upon probing  $\geq 50\%$ .

Results in the undiagnosed diabetics mirrored the findings in the IFG groups, showing that severe clinical periodontal inflammation was associated with undiagnosed diabetes after adjusting for all covariates. (OR=1.5, 95% CI: 1.0-2.2). (**Table 10**)

To further explore the relationship, between prediabetes and periodontal inflammation, we performed four additional analyses using markers of systemic inflammation (serum antibody levels to the periodontal pathogens *Porphyromonas gingivalis* and *Actinobacillus actinmycetemcomitans*), and

markers of localized inflammation (gingival crevicular fluid levels of IL-1 $\beta$  (GCF-IL-1 $\beta$ ) and gingival crevicular fluid levels of prostaglandin (PG-E2) (**Tables 11-13**). However, no significant association was observed between pre-diabetes or undiagnosed diabetes and any of those inflammation markers.

**Table 4-Baseline characteristics of 6,138 middle-aged adults with periodontal exams according to glycemia status. ARIC Dental Study, 1996 – 1998**

	Normal Glucose	Impaired Glucose Tolerance	Impaired Fasting Glucose	Undiagnosed Diabetes	P value
N	2,154	1,572	1307	1105	
Male sex (%)	61.8	62.0	58.5	57.6	<0.0001
Age (years)	61.5±5.6	63.3±5.6	61.3±5.6	62.8±5.4	<0.0001
African American (%)	11.0	12.0	16.3	23.9	<0.0001
Education <=12 Years (%)	56.2	46.0	56.0	50.6	<0.0001
Smoking Status (%) - Current	12.7±0.3	10.1±0.3	14.9±0.4	12.7±0.3	0.0082
Sports Index	2.6±0.8	2.5±0.8	2.6±0.8	2.5±0.8	<0.0001
Total Calorie Intake (Kcal/day)	1,578±652	1,587±603	1,637±673	1,608±690	0.1253
Body mass index (kg/m <sup>2</sup> )	26.8±4.3	28.9±4.8	28.9±5.2	29.4±5.9	<0.0001
Waist Circumference (cm)	95.9±12.6	102.5±13.6	102.6±13.2	103.7±14.2	<0.0001
Hypertension (%)	30.1	48.8	39.2	51.8	<0.0001
Previous Cardiovascular Disease (%)	4.9	6.0	5.5	6.7	0.3020
Family History of Diabetes (%)	11.5	15.3	12.1	14.8	0.013
High Density Lipoprotein (mmol/L)	1.4±0.5	1.3±0.4	1.2±0.4	1.3±0.3	<0.0001

Characteristics are statistically different if  $p < 0.05$  using ANOVA tests for continuous variables and  $\chi^2$  for categorical variables

Data are mean  $\pm$  SD or percent.

Normal glucose=FG<100mg/dL & 2hrGTT<140mg/dL & no diabetes

Impaired Glucose Tolerance=2hr glucose of 140-199mg/dL & no diabetes

Impaired fasting glucose=FG of 100-125mg/dL & 2hr GTT<140mg/dL & no diabetes

Undiagnosed diabetes=FG>125mg/dL, or 2hr GTT>199mg/dL & no diabetes diagnosis

**Table 5-Baseline characteristics of 6,138 Visit 4 participants receiving dental examinations excluding diagnosed diabetic subjects.**

	Category I	Category II	Category III	Category IV	Category V	P value
N	891	913	1,134	2,443	757	
Male sex (%)	73.1	63.3	52.1	50.3	40.4	<0.0001
Age (years)	62.2±5.6	62.3±5.6	62.4±5.5	62.3±5.6	62.7±5.4	0.3393
African American (%)	29.5	23.5	11.1	10.7	30.5	<0.0001
Education ≤12 Years (%)	53.9	48.3	63.6	52.3	44.9	<0.0001
Smoking Status (%)	10.7±0.3	8.5±0.3	14.4±0.4	12.2±0.3	17.6±0.3	<0.0001
Current Sports Index	2.5±0.6	2.5±0.7	2.7±0.8	2.6±0.6	2.4±0.7	<0.0001
Total Calorie Intake (Kcal/day)	1548±667	1556±607	1564±588	1627±641	1739±769	<0.0001
Body mass index (kg/m <sup>2</sup> )	28.3±3.6	28.5±4.5	27.5±5.6	28.4±4.4	29.0±5.6	<0.0001
Waist Circumference (cm)	101.9±12.6	104.5±13.6	102.6±11.2	103.8±12.2	107±13.5	<0.0001
Hypertension (%)	46.6	43.4	32.7	39.9	49.3	<0.0001
Previous Cardiovascular Disease (%)	4.5	4.6	5.6	5.2	6.9	0.0096
Family History of Diabetes (%)	12.2	13.4	12.4	10.8	13.2	0.11
High Density Lipoprotein (mmol/L)	1.4±0.5	1.6±0.5	1.3±0.6	1.4±0.5	1.7±0.3	<0.0001
Mean Fasting Glucose (mg/dL)	100.0±13.2	102.4±13.6	100.0±14.6	101.6±14.1	108.1±13.2	<0.0001
Mean 2hr Glucose Tolerance Test (mg/dL)	135.0±17.1	141.1±18.4	127.4±19.2	135.9±20.0	143.4-±18.6	<0.0001

Characteristics are statistically different if p<0.05 (ANOVA tests for continuous variables and  $\chi^2$  for categorical variables), Category I=probing depth (PD) ≤3mm, bleeding upon probing ≤10% (reference

category), Category II=probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $>10\%$ , Category III=one or more sites with PD $\geq 4$ mm, bleeding upon probing  $\leq 10\%$ , Category IV=one or more sites with PD $\geq 4$ mm, bleeding upon probing  $>10\%$  and  $<50\%$ , Category V=one or more sites with PD $\geq 4$ mm, bleeding upon probing  $\geq 50\%$ . Data are mean  $\pm$  SD or percent.

**Table 6-Prevalence(%) with normal glycemia, IGT, IFG, and undiagnosed diabetes by clinical category in 6, 138 ARIC Dental Study participants**

	N	Category I (%)	Category II (%)	Category III (%)	Category IV (%)	Category V (%)
Normal	2,154	14.2	11.6	16.9	42.9	9.7
IGT	1,572	11.1	20.2	20.0	29.5	19.1
IFG	1,307	8.7	15.3	18.4	36.1	21.4
Undiagnosed Diabetes	1105	13.6	18.6	20.8	29.0	18.1

P-value was  $P < 0.0001$  using a  $\chi^2$  test

Category I=probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $\leq 10\%$  (reference category)

Category II=probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $>10\%$

Category III=one or more sites with PD $\geq 4$ mm, bleeding upon probing  $\leq 10\%$

Category IV=one or more sites with PD $\geq 4$ mm, bleeding upon probing  $>10\%$  and  $<50\%$

Category V=one or more sites with PD $\geq 4$ mm, bleeding upon probing  $\geq 50\%$

Normal glucose=FG $<100$ mg/dL & 2hrGTT $<140$ mg/dL & no diabetes

Impaired Glucose Tolerance=2hr glucose of 140-199mg/dL & no diabetes

Impaired fasting glucose=FG of 100-125mg/dL & 2hr GTT $<140$ mg/dL & no diabetes

Undiagnosed diabetes=FG $>125$ mg/dL, or 2hr GTT $>199$ mg/dL & no diabetes diagnosis

**Table 7- Prevalence (%) of normoglycemia, IGT, IFG, and undiagnosed diabetes by inflammatory marker in 5,109 ARIC Dental Study participants without diagnosed diabetes**

	N	Antibody to P. gingivalis		Antibody to A.a.		GCF levels of IL-1 $\beta$		GCF levels of PG-E2	
		High	Low	High	Low	High	Low	High	Low
Normal	4054	6.6	93.4	5.0	95.0	3.5	97.5	6.6	93.4
IGT	620	8.1	91.9	5.6	94.4	3.7	96.2	7.0	93.0
FG	314	7.8	92.2	4.8	95.2	3.5	97.5	5.7	94.3
Undiagnosed	121	5.7	94.3	4.9	95.1	3.7	96.2	5.4	94.6
P- value		0.45		0.34		0.09		0.59	

All P-values used  $\chi^2$  tests for each inflammatory marker

Normal glucose=FG<100mg/dL & 2hrGTT<140mg/dL & no diabetes

Impaired Glucose Tolerance=2hr glucose of 140-199mg/dL & no diabetes

Impaired fasting glucose=FG of 100-125mg/dL & 2hr GTT<140mg/dL & no diabetes

Undiagnosed diabetes=FG>125mg/dL, or 2hr GTT>199mg/dL & no diabetes diagnosis

High *Porphyromonas gingivalis* antibody levels at  $\geq 78.93$  EU (highest quartile)

High *Actinobacillus actinmycetemcomitans* antibody levels  $\geq 144$  EU (highest quartile)

High GCF- IL-1 $\beta$  levels at  $\geq 146$ ng/mL (highest quartile)

High GCF PG-E2 levels  $\geq 239$ ng/mL (highest quartile)

Low= lower 3 quartiles for all markers

**Table 8- Odds ratios for Impaired Glucose Tolerance in 6, 138 ARIC Dental Study participants without diagnosed diabetes by Category of Clinical Periodontal Inflammation**

	Category I	Category II	Category III	Category IV	Category V
Model 1	1.0	1.2 (0.9-1.4)	0.8 (0.6-1.0)	1.0 (0.8-1.3)	1.3 (1.0-1.7)
Model 2	1.0	1.2 (0.9-1.5)	0.8 (0.7-1.1)	1.0 (0.8-1.3)	1.2 (0.9-1.6)
Model 3	1.0	1.1 (0.8-1.4)	0.9 (0.7-1.2)	1.0 (0.8-1.3)	1.3 (0.9-1.7)
Model 4	1.0	1.0 (0.7-1.3)	0.8 (0.6-1.1)	0.9 (0.7-1.2)	1.2 (0.8-1.7)
Model 5	1.0	1.1 (0.8-1.4)	0.8 (0.6-1.1)	0.9 (0.7-1.1)	1.0 (0.7-1.3)

Model 1- unadjusted

Model 2- adjusted for sex, age, race, and education

Model 3- adjusted for lifestyle covariates –(sex, age, race, education, physical activity)

Model 4-adjusted for lifestyle covariates and waist circumference

Model 5- fully adjusted (for sex, age race, education, smoking, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol, total caloric intake)

Category I=probing depth (PD) ≤3mm, bleeding upon probing ≤10% (reference category)

Category II=probing depth (PD) ≤3mm, bleeding upon probing >10%

Category III=one or more sites with PD≥4mm, bleeding upon probing ≤10%

Category IV=one or more sites with PD≥4mm, bleeding upon probing>10% and <50%

Category V=one or more sites with PD≥4mm, bleeding upon probing ≥50%



**Table 9- Odds ratios for Impaired Fasting Glucose in 6, 138 ARIC Dental Study participants without diagnosed diabetes by Category of Clinical Periodontal Inflammation**

	Category I	Category II	Category III	Category IV	Category V
Model 1	1.0	1.4 (1.0-1.8)	1.3 (1.0-1.6)	1.5 (1.2-1.9)	2.1 (1.6-2.8)
Model 2	1.0	1.2 (0.9-1.6)	1.1 (0.8-1.5)	1.3 (1.0-1.6)	1.6 (1.1-2.1)
Model 3	1.0	1.2 (0.8-1.7)	1.2 (0.8-1.6)	1.3 (1.0-1.7)	1.5 (1.1-2.2)
Model 4	1.0	1.1 (0.8-1.6)	1.1 (0.8-1.6)	1.2 (0.9-1.6)	1.4 (1.0-2.0)
Model 5	1.0	1.2 (0.8-1.6)	1.1 (0.8-1.5)	1.3 (1.0-1.7)	1.5 (1.1-2.1)

Model 1- unadjusted

Model 2- adjusted for sex, age, race, and education

Model 3- adjusted for lifestyle covariates –(sex, age, race, education, physical activity)

Model 4-adjusted for lifestyle covariates and waist circumference

Model 5- fully adjusted (for sex, age race, education, smoking, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol, total caloric intake)

Category I=probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $\leq 10\%$  (reference category)

Category II=probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $>10\%$

Category III=one or more sites with PD $\geq 4$ mm, bleeding upon probing  $\leq 10\%$

Category IV=one or more sites with PD $\geq 4$ mm, bleeding upon probing  $>10$  and  $<50\%$

Category V=one or more sites with PD $\geq 4$ mm, bleeding upon probing  $\geq 50\%$

**Table- 10-Odds ratios for undiagnosed diabetes in 6, 138 ARIC Dental Study participants without diagnosed diabetes by Category of Clinical Periodontal Inflammation**

	Category I	Category II	Category III	Category IV	Category V
Model 1	1.0	1.3 (1.0-1.7)	0.7 (0.6-1.0)	1.1 (0.8-1.3)	2.0 (1.4-2.9)
Model 2	1.0	1.2 (1.0-1.8)	0.9 (0.7-1.3)	1.2 (0.9-1.6)	1.9 (1.3-2.7)
Model 3	1.0	1.2 (0.9-1.7)	0.9 (0.7-1.3)	1.3 (1.0-1.7)	1.9 (1.3-2.7)
Model 4	1.0	1.2 (0.9-1.7)	0.9 (0.7-1.3)	1.1 (0.9-1.5)	1.7 (1.2-2.4)
Model 5	1.0	1.2 (0.9-1.8)	0.9 (0.6-1.3)	1.1 (0.8-1.5)	1.5 (1.0-2.2)

Model 1- unadjusted

Model 2- adjusted for sex, age, race, and education

Model 3- adjusted for lifestyle covariates –(sex, age, race, education, physical activity)

Model 4-adjusted for lifestyle covariates and waist circumference

Model 5- fully adjusted (for sex, age race, education, smoking, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol, total caloric intake)

Category I=probing depth (PD) ≤3mm, bleeding upon probing ≤10% (reference category)

Category II=probing depth (PD) ≤3mm, bleeding upon probing >10%

Category III=one or more sites with PD≥4mm, bleeding upon probing ≤10%

Category IV=one or more sites with PD≥4mm, bleeding upon probing >10% and <50%

Category V=one or more sites with PD≥4mm, bleeding upon probing ≥50%

**Table 11- Odds ratios for impaired Glucose in 5,109 ARIC Dental Study participants without diagnosed diabetes by Inflammatory Markers for Periodontal Inflammation**

	Antibody to P. gingivalis (high vs. low)	Antibody to A.a. (high vs. low)	GCF levels of IL- 1 $\beta$ (high vs. low)	GCF levels of PG-E2 (high vs. low)
Model 1	1.2 (1.0-1.4)	1.2 (1.0-1.4)	1.1 (0.9-1.4)	1.1 (0.9-1.3)
Model 2	1.2 (1.0-1.4)	1.1 (0.9-1.3)	1.1 (0.9-1.4)	1.2 (1.0-1.4)
Model 3	1.1 (0.9-1.4)	1.1 (0.9-1.3)	1.1 (0.9-1.3)	1.2 (1.0-1.4)
Model 4	1.1 (0.9-1.4)	1.1 (0.9-1.3)	1.1 (0.9-1.3)	1.1 (1.0-1.4)
Model 5	1.2 (0.9-1.4)	1.1 (0.9-1.3)	1.1 (0.9-1.4)	1.1 (0.9-1.3)

Model 1- unadjusted

Model 2- adjusted for sex, age, race, and education

Model 3- adjusted for lifestyle covariates –(sex, age, race, education, physical activity)

Model 4-adjusted for lifestyle covariates and waist circumference

Model 5- fully adjusted (for sex, age race, education, smoking, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol, total caloric intake)

Category I=probing depth (PD)  $\leq$ 3mm, bleeding upon probing  $\leq$ 10% (reference category)

Category II=probing depth (PD)  $\leq$ 3mm, bleeding upon probing  $>$ 10%

Category III=one or more sites with PD $\geq$ 4mm, bleeding upon probing  $\leq$ 10%

Category IV=one or more sites with PD $\geq$ 4mm, bleeding upon probing  $>$ 10% and  $<$ 50%

Category V=one or more sites with PD $\geq$ 4mm, bleeding upon probing  $\geq$ 50%

High *Porphyromonas gingivalis* antibody levels at  $\geq$ 78.93 EU (highest quartile)

High *Actinobacillus actinmycetemcomitans* antibody levels  $\geq$ 144 EU (highest quartile)

High GCF- IL-1 $\beta$  levels at  $\geq$ 146ng/mL (highest quartile)

High GCF PG-E2 levels  $\geq$ 239ng/mL (highest quartile)

Low= lower 3 quartiles for all markers

**Table 12- Odds ratios for Impaired Fasting Glucose in 5,109 ARIC Dental Study participants without diagnosed diabetes by Systemic Markers for Periodontal Inflammation**

	Antibody to P. gingivalis (high vs. low)	Antibody to A.a. (high vs. low)	GCF levels of IL- 1 $\beta$ (high vs. low)	GCF levels of PG-E2 (high vs. low)
Model 1	1.1 (0.9-1.3)	1.0 (0.8-1.2)	1.0 (0.8-1.2)	0.9 (0.8-1.1)
Model 2	1.0 (0.8-1.2)	0.9 (0.8-1.1)	1.0 (0.8-1.2)	1.0 (0.8-1.2)
Model 3	1.0 (0.9-1.2)	0.9 (0.7-1.1)	0.9 (0.8-1.2)	1.0 (0.8-1.2)
Model 4	1.0 (0.8-1.2)	0.9 (0.7-1.1)	0.9 (0.7-1.1)	1.0 (0.8-1.2)
Model 5	1.0 (0.8-1.2)	0.9 (0.8-1.1)	1.0 (0.8-1.2)	0.9 (0.7-1.1)

Model 1- unadjusted

Model 2- adjusted for sex, age, race, and education

Model 3- adjusted for lifestyle covariates –(sex, age, race, education, physical activity)

Model 4-adjusted for lifestyle covariates and waist circumference

Model 5- fully adjusted (for sex, age race, education, smoking, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol, total caloric intake)

Category I=probing depth (PD)  $\leq$ 3mm, bleeding upon probing  $\leq$ 10% (reference category)

Category II=probing depth (PD)  $\leq$ 3mm, bleeding upon probing  $>$ 10%

Category III=one or more sites with PD $\geq$ 4mm, bleeding upon probing  $\leq$ 10%

Category IV=one or more sites with PD $\geq$ 4mm, bleeding upon probing  $>$ 10% and  $<$ 50%

Category V=one or more sites with PD $\geq$ 4mm, bleeding upon probing  $\geq$ 50%

High *Porphyromonas gingivalis* antibody levels at  $\geq$ 78.93 EU (highest quartile)

High *Actinobacillus actinmycetemcomitans* antibody levels  $\geq$ 144 EU (highest quartile)

High GCF- IL-1 $\beta$  levels at  $\geq$ 146ng/mL (highest quartile)

High GCF PG-E2 levels  $\geq$ 239ng/mL (highest quartile)

Low= lower 3 quartiles for all markers

**Table 13- Odds ratios for Undiagnosed Diabetes by Systemic Markers for Periodontal Inflammation**

	Antibody to P. gingivalis (high vs. low)	Antibody to A.a. (high vs. low)	GCF levels of IL- 1 $\beta$ (high vs. low)	GCF levels of PG-E2 (high vs. low)
Model 1	1.2 (1.0-1.5)	1.2 (1.0-1.4)	1.0 (0.9-1.2)	0.9 (0.7-1.1)
Model 2	0.9 (0.8-1.1)	1.0 (0.8-1.2)	1.2 (1.0-1.4)	0.9 (0.8-1.1)
Model 3	1.0 (0.8-1.2)	1.0 (0.8-1.2)	1.1 (0.9-1.4)	0.9 (0.8-1.1)
Model 4	0.9 (0.8-1.2)	1.0 (0.8-1.2)	1.1 (0.9-1.3)	0.9 (0.7-1.1)
Model 5	0.9 (0.7-1.1)	0.9 (0.7-1.1)	1.1 (0.9-1.3)	0.8 (0.7-1.1)

Model 1- unadjusted

Model 2- adjusted for sex, age, race, and education

Model 3- adjusted for lifestyle covariates –(sex, age, race, education, physical activity)

Model 4-adjusted for lifestyle covariates and waist circumference

Model 5- fully adjusted (for sex, age race, education, smoking, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol, total caloric intake)

Category I=probing depth (PD)  $\leq$ 3mm, bleeding upon probing  $\leq$ 10% (reference category)

Category II=probing depth (PD)  $\leq$ 3mm, bleeding upon probing  $>$ 10%

Category III=one or more sites with PD $\geq$ 4mm, bleeding upon probing  $\leq$ 10%

Category IV=one or more sites with PD $\geq$ 4mm, bleeding upon probing  $>$ 10% and  $<$ 50%

Category V=one or more sites with PD $\geq$ 4mm, bleeding upon probing  $\geq$ 50%

High *Porphyromonas gingivalis* antibody levels at  $\geq$ 78.93 EU (highest quartile)

High *Actinobacillus actinomycetemcomitans* antibody levels  $\geq$ 144 EU (highest quartile)

High GCF- IL-1 $\beta$  levels at  $\geq$ 146ng/mL (highest quartile)

High GCF PG-E2 levels  $\geq$ 239ng/mL (highest quartile)

Low= lower 3 quartiles for all markers

## Discussion

In this cross-sectional analysis, we found clinical periodontal measures for inflammation were associated with the likelihood of impaired fasting glucose. This association seemed to hold true for individuals with only slight bleeding upon probing, with deeper probing measurements, or more severe gingival bleeding upon probing. However, a dose response relationship was not observed with increasing severity of periodontal inflammation. These results were consistent with the clinical implications since probing depths give a measure of prior periodontal attachment loss, while bleeding upon probing assesses current inflammation. A periodontal exam that combines probing depth and bleeding scores gives an accurate assessment of prior and current periodontal status, and both are used together in the examination and diagnosis of dental patients in the clinical setting.

The association for clinical periodontal inflammation may even appear mildly protective for undiagnosed diabetics in Category III periodontitis, though this association did not appear significant (OR=0.9, 95 % CI: 0.6-1.3). This may be explained by the means of assessment of inflammation, since even though the probing depth has increased when comparing Categories III to I, the bleeding score is the same from both groups ( $\leq 10\%$  bleeding upon probing is assigned for both categories). Bleeding upon probing may give a better picture in the biologic pathway of active, or current periodontal inflammation. Probing measurements

represent attachment loss from prior periodontal disease exposure, thus giving a clinical picture of past history of lost supporting periodontal tissues including bone and periodontal ligament fibers around remaining teeth. No information regarding current or prior periodontal treatment was available in this dataset, which may have helped to explain active versus prior periodontal inflammation.

A subsidiary analysis of baseline characteristics of 4,864 individuals available at visit 4, but excluded from the periodontal examination, was performed (results not shown). The individuals excluded from our primary analysis had a higher proportion of African-Americans (39% vs. 24%), smokers (19% vs. 13%), increased caloric intake (1773 kcal/day vs. 1587kcal/day) and higher body mass-indices ( $31\text{kg/m}^2$  vs.  $28.9\text{kg/m}^2$ ). These aforementioned characteristics are known risk factors for diabetes. It is possible that exclusion of these individuals from the analysis may have resulted in an underestimated risk of pre-diabetes. Additionally, 15 % (n=1,478) of the visit 4 participants were edentulous. If we assume that tooth loss is a surrogate for severe periodontal disease status, then it is possible a large proportion of individuals with prior exposure to severe periodontal inflammation were not available for analysis, also resulting in an underestimated risk of pre-diabetes.

A stronger association of periodontal disease with fasting glucose than with glucose tolerance tests was observed. This may be explained by the less than 100% concordance rate between these two tests.<sup>49</sup> In clinical practice, when

there is disparity between the test, the test whose result is higher should be repeated.<sup>49</sup> Repeat tests results for confirmation of classification of diabetic status were not available in this ARIC dataset.

This study is novel by combining both clinical and systemic measures specific to periodontal inflammation to correlate with pre-diabetes. This approach was used to assess cardiovascular disease as an outcome.<sup>54</sup> However, unlike in those cardiovascular studies, our study did not show a significant association between systemic inflammatory mediators and pre-diabetes. Our study suggested that the association of periodontal inflammation with pre-diabetes was not the same as the association with the risk of cardiovascular disease.<sup>50, 51</sup> It indicated that the biologic pathway of periodontal inflammation is different when comparing impaired glucose tolerance and prediabetes with cardiovascular disease. While serum antibodies levels to periodontal pathogens *Porphyromonas gingivalis* and *Actinobacillus actinmycetemcomitans* can show prior exposure to periodontal inflammation, these levels did not show an association with impaired glucose, elevated fasting glucose, or undiagnosed diabetes in our fully adjusted models.

While IL-1 $\beta$  and PG-E2 levels have been shown to be elevated in presence of both periodontal disease and diabetes in other studies, our data did not show this association. The few mechanistic studies looking at markers for inflammation common to the pathogenesis of periodontal disease and diabetic



status, included type 2 diabetic patients in very small studies to conclude that IL-1 $\beta$  may be associated with both diseases.<sup>26,43,44</sup> Inclusion of individuals with diabetes may significantly alter the inflammatory profile, and those studies may have displayed reverse causality, where diabetes, not periodontal disease, increased inflammatory marker levels. Even though we excluded type 2 diabetes individuals from our data set, our sample size of 5109 subjects with laboratory assays still consisted of a large number of individuals for analysis. While clear association could not be seen with IL-1 $\beta$  and PG-E2 levels in gingival crevicular fluid with impaired glucose levels, our study indicated additional inflammatory markers might need to be examined to better understand this association. A more complex, or non-linear association may be possible, as expected with other inflammatory mediators.

By excluding individuals with diagnosed diabetes in the cross sectional analysis, we reduce the possibility of reverse causality of diabetes causing periodontal inflammation. Unlike the majority of previous studies that looked at the association of periodontal disease with diabetes, this study is strengthened by leaving this group out, in order to see if pre-diabetes status is associated with periodontal inflammation. Individuals with diabetes also have widespread systemic abnormalities in the immune response, which can create an over-estimate or residual confounding for the association of periodontal disease with diabetes.

The serum levels for inflammatory mediators used the highest quartile to define high vs. low levels, as in other studies that assessed the association of periodontal disease with cardiovascular disease risk.<sup>51</sup> It is possible that the highest quartile may represent a unique population with the possibility for residual confounding. This study used a similar cut-point, thus may have overestimated the association of periodontal inflammation with pre-diabetes. Additionally, genetic risk factors known to affect diabetic status were not available in this dataset, and were not included in these models.

The potential for selection bias exists, since not all Visit 4 participants were included in the analysis. It is possible that the 15% (n=1,748) of edentulous participants excluded from this analysis had a history of the most advanced periodontal status, with resulting prior tooth loss and subsequent edentulism. This study is not generalizable to individuals missing all their dentition. The periodontal status of the 13% (N=1,515) that refused the dental exam remains unknown.

Using one time measure of fasting glucose may not be reliable. Repeat test results for confirmation of classification of diabetic status is recommended in clinical practice, but was not available in this dataset.

## Conclusions

This study suggested clinical periodontal inflammation was cross-sectionally associated with impaired fasting glucose. An increase in bleeding upon probing appears to be cross-sectionally associated with impaired fasting glucose. Since even minimal bleeding upon probing in this study was associated with prevalent pre-diabetes and undiagnosed diabetes, a strong case may be made to support patient education for prevention of periodontal disease and study the effect of prevention of even mild periodontal inflammation on prediabetes and diabetes.

With an association of clinical measures of periodontal inflammation with pre-diabetes, an assessment of risk of impaired fasting glucose may be performed easily in the dental office. This requires a dental exam commonly and currently performed by dental providers. The use of immunoassays, which are costly, and technically demanding, may not be necessary to define the relationship of periodontal disease with pre-diabetes.

Presumably, the prevention of gingival inflammation may moderately reduce the risk of diabetes onset. The reduction of periodontal disease, and then diabetes, has potential to slow the growing epidemic proportions of both diseases. This study contributes to the sparse evidence for the association of periodontal association with pre-diabetes.

## Chapter 3

# The prospective association of periodontal disease and the risk of type 2 diabetes

### Abstract

Though the bi-directionality of periodontal disease and diabetes is widely discussed, evidence of periodontal disease and subsequent risk of diabetes is sparse. Using data from the Atherosclerosis Risk in Communities (ARIC) Study, we tested the hypothesis that exposure to periodontal inflammation, using clinical exam evidence, systemic inflammatory markers and local inflammatory markers, predicts the subsequent occurrence of incident type 2 diabetes. Of the total 5,819 eligible participants at baseline (ARIC Visit 4), 1,967 individuals developed incident type 2 diabetes after a mean of 13.8 years of follow-up. Incident diabetes was assessed with yearly telephone interviews and self-reports from study participants.

In multivariable analyses using the Cox proportional hazards model, when compared to Category I (probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $\leq 10\%$ ), the hazard ratio of incident diabetes was the highest with early periodontal clinical measures of inflammation as found in Category II (probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $> 10\%$ ) (HR=1.4, 95%CI: 1.1-1.7,  $p < 0.001$ ) after adjustment for sex, age, race, education level, smoking status,

physical activity, total caloric intake, waist circumference, hypertension, previous cardiovascular disease, family history of diabetes, and HDL cholesterol levels. Compared with individuals in Category I, with minimal bleeding and probing measures, the hazard of incident diabetes appears to be 1.2 times higher (95% CI: 1.0 – 1.4,  $p < 0.001$ ) in adults with moderate clinical periodontal inflammation (Category IV-one or more sites with  $PD \geq 4$ mm, bleeding upon probing  $> 10\%$  &  $< 50\%$ ) and 1.3 times higher (95% CI: 1.0- 1.6,  $P < 0.001$ ) in adults with advanced clinical periodontal inflammation (Category V- one or more sites with  $PD \geq 4$ mm, bleeding upon probing  $\geq 50\%$ ). This data supports the hypothesis that periodontal exposure increases the risk of subsequent incident diabetes.

## **Introduction**

A two-way relationship between type 2 diabetes and periodontal disease has been discussed in the literature, as a clear association between hyperglycemia and severity of periodontal disease has been shown.<sup>52</sup> The mechanism of this relationship has not been completely understood, but physiological models propose an immunologic response, and inflammation appears common to the pathogenesis of both diseases.<sup>19</sup> Evidence to help define the directionality of periodontal disease and risk of type 2 diabetes are important to understand possible mechanisms common to both diseases. Such studies are lacking in the literature, despite discussion of the bidirectional relationship for almost the past 20 years. While a preponderance of literature shows the effect of diabetes on periodontal inflammation, the evidence supporting the effect of

periodontal disease on the risk of incident diabetes is lacking. The consensus report from the Joint European Federation of Periodontology and the American Academy of Periodontology recommended longitudinal designs, large cohorts, and inclusion of clinical and immunologic biomarkers to help define the impact of periodontal inflammation on incident diabetes.<sup>53</sup>

Systemic inflammation has emerged as a risk factor for type 2 diabetes, but the contribution of periodontal inflammation to diabetes onset is unknown. Both diabetes and periodontal disease have been found to result in an elevation of inflammatory cytokines as a host response. Gram-negative bacteria found in periodontal disease have been found to result in elevated levels of these cytokines, such as Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in both the gingival crevicular fluid and in peripheral blood in individuals with diabetes and periodontal disease. Those with diabetes and advanced periodontal disease had two-fold higher levels of PGE<sub>2</sub> and Interleukin-, 1 $\beta$  (IL- 1 $\beta$ ) when compared to those with diabetes and milder forms of periodontal disease.<sup>27</sup>

Evidence to help define the directionality of periodontal disease and risk of type 2 diabetes are important to understand possible mechanisms common to both diseases. Such studies are lacking in the literature, despite discussion of the bidirectional relationship for almost the past 20 years. We hypothesized that exposure to periodontal inflammation, (using clinical exam evidence, systemic inflammatory markers and local inflammatory markers), predicts the subsequent

occurrence of incident type 2 diabetes. Our study uniquely looks at comprehensive clinical measures of periodontal inflammation (bleeding upon probing and full mouth probing depths), systemic markers for prior exposure to periodontal inflammation (serum antibodies to the periodontal pathogens (*Porphyromonas gingivalis* and *Actinobacillus actinmycetemcommitans*), and local biomarkers for periodontal inflammation (gingival crevicular fluid levels of IL-1 $\beta$  and PG-E2) in a cohort followed for approximately 14 years to assess subsequent risk of incident type 2 diabetes.

## **Methods**

### ***Study Population***

The Atherosclerosis Risk in Communities (ARIC) Study is a community-based prospective cohort of 15,792 middle-aged adults from four U.S. communities. The first examination of participants (Visit 1) took place during 1987–1989, with three follow-up visits taking place: each approximately every 3 years. The Dental ARIC study, an ancillary study, funded by the National Institute of Dental and Craniofacial Research (NIDCR), was conducted during ARIC Visit 4 in 1996 through 1998 and is cross-sectional in design. The Dental ARIC consisted of an oral examination, collection of serum, and interviews. Of those 15,792 ARIC cohort members examined at baseline (1987 to 1989), responders to a dental screening interview were selected. Respondents with no teeth or a medical contraindication to probing were excluded, while some refused the

dental exam. In addition, participants with type 2 diabetes, missing demographic data, missing serum samples, and unreadable samples were excluded. The final analysis included data from 5109 participants. (**Figure 4** in Chapter 2 showed the participants available at baseline for this analysis,)

### ***Periodontal Disease***

Clinical assessments of periodontal inflammation were defined by two assessments: bleeding upon probing and periodontal pockets (rounded down to the nearest mm). This definition is consistent with the standard of care in assessing the clinical periodontal status. (**See Appendix- Figure 17**) Using these two parameters, participants were classified into 5 categories: <sup>46</sup>

- I) probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $\leq 10\%$
- II) probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $> 10\%$
- lii) one or more sites with PD  $\geq 4$ mm, bleeding upon probing  $\leq 10\%$
- IV) one or more sites with PD  $\geq 4$ mm, bleeding upon probing  $> 10\%$  &  $< 50\%$
- V) one or more sites with PD  $\geq 4$ mm, bleeding upon probing  $\geq 50\%$

Serum markers of prior periodontal disease exposure were defined by serum IgG antibodies to the periodontal pathogens *Porphyromonas gingivalis* and serum IgG antibodies to *Actinobacillus actinmycetemcommitans*. These variables were measured as the level of antibody response to the periodontal pathogen *Porphyromonas gingivalis* and *Actinobacillus actinmycetemcommitans*



in Elisa units (EU). Using the upper quartile as the cut-point, the high antibody group was compared to the low antibody group (lower three quartiles).

*Porphyromonas gingivalis* antibody levels were considered high at  $\geq 78.93$  EU, and *Actinobacillus actinmycetemcomitans* antibody levels were considered high at  $\geq 144$  EU. The use of the upper quartile for assigning the high antibody level group has been used on other studies.<sup>51</sup> The normal antibody level in periodontal health for these periodontal pathogens has not yet been established.

Local inflammatory markers of periodontal disease were assessed using gingival crevicular fluid levels of IL-1 $\beta$  (GCF- IL-1 $\beta$ ) and gingival crevicular fluid levels of prostaglandin (PG-E2). The variable GCF- IL-1 $\beta$  was measured as the level of gingival crevicular fluid units (ng/mL). Using the upper quartile as the cut-point, subjects were considered to have high levels of GCF- IL-1 $\beta$  levels at  $\geq 146$  ng/mL. The variable PG-E2 was also measured as the level of gingival crevicular fluid units (ng/mL) and a dichotomous variable (high/low) was used. Subjects were considered to have elevated levels of PG-E2 levels at  $\geq 239$  ng/mL using the upper quartile cut-point. Similarly to the antibody levels to *P.g* and *A.a*, normal levels of IL-1 $\beta$  and PG-E2 in periodontal health have not been established.

All clinical periodontal measures, as well as serum and gingival crevicular samples were measured at VIsit 4 (1996 through 1998).

### ***Prevalent Type 2 Diabetes***

The ARIC visit 4 individuals were classified as having a diabetes diagnosis if any of the following criteria were met; self-report of current use of medication for diabetes or blood sugar; or a positive response to the question “Has a doctor ever told you that you had diabetes (sugar in the blood)?”. Undiagnosed diabetic individuals were classified as having fasting glucose of at least 7.0mmol/L (126mg/dL); non-fasting glucose of at least 11.1mmol/L (200mg/dL), but no doctor diagnosis of diabetes and no self-report of anti-diabetic medication. These ARIC definitions were based on the 1997 American Diabetes Association criteria available at the time of the ARIC Visit 4.

### ***Incident Type 2 Diabetes***

Individuals from baseline Visit 4 were telephoned yearly and were classified as having diabetes if answering positive to either current use of anti-diabetic medication or having been “told by a doctor that they have diabetes or sugar in the blood”. Persons classified as having diabetes at baseline were excluded. This dataset includes self-reports that were obtained until the end date of April 2011.

### ***Other Variables***

Covariates measured at the visit 4 baseline included sex, age, race, education, smoking, physical activity, total caloric intake, BMI, waist

circumference, hypertension, previous cardiovascular disease, family history of diabetes, and high density lipoprotein levels. Information on age, sex, race, smoking, total caloric intake, education level and family history of diabetes were based on self-report. BMI (weight in kilograms divided by the square height in meters) and waist to hip ratio (in centimeters) were measured with standard procedures.<sup>47</sup> Prevalent cardiovascular disease was based on self-report, ARIC clinical exam, or hospital records. The physical activity was assessed using a modified version of the questionnaire developed by Baecke and colleagues, from which a sport index was derived, ranging from 1 (lowest) to 5 (highest).<sup>48</sup> HDL cholesterol levels were measured after dextran-magnesium precipitation. The education levels, however were measured earlier, at visit 1 (1978-1989), and then were dichotomized into  $\leq 12$  years or  $>12$  years of education.

### ***Data Analysis***

All subjects with diagnosed or undiagnosed diabetes at the baseline (visit 4) were excluded. Therefore, 5109 participants were included in the final analysis. Individuals from baseline visit 4 were telephoned yearly and were classified as having diabetes if answering positive to either current use of anti-diabetic medication or having been “told by a doctor that they have diabetes or sugar in the blood”. For participants without diabetes, study time was calculated from baseline Visit 4 to the last follow-up date. Individuals who died were censored at the date of death.

Baseline characteristics of participants were described using means and frequencies of each potential confounder for each category of clinical periodontal inflammation (Category I-V- **Table 14**). ANOVA and  $\chi^2$  analyses were used to assess the statistical differences across the 5 categories. Similar descriptive statistics were also performed for 3 categories of bleeding upon probing (mild BOP= bleeding upon probing  $\leq 10\%$  (reference category), moderate BOP= bleeding upon probing 11-49%, Severe BOP= bleeding upon probing  $\geq 50\%$ ) (**Table 15**).

To explore the relationship between periodontal disease and incident diabetes, five models were constructed to adjust for demographic variables (sex, age, race, and education), lifestyle covariates (physical activity), waist circumference, and medical history (cardiovascular disease, family history of diabetes, hypertension, high density lipoprotein levels, and total caloric intake).

Time to incident diabetes was assessed over a mean of 13.84 years of follow-up. Kaplan-Meier survival analysis curves were plotted and incidence rates (1000 person-years) were calculated for periodontal disease (Categories I-V), antibody levels to the pathogens *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*, and serum gingival crevicular fluid levels of IL-1 $\beta$  (GCF- IL-1 $\beta$ ) and prostaglandin (PG-E2). Log-rank tests were performed for categories of periodontal disease (I-V) and for categories of bleeding upon probing (mild/moderate/severe). Cox proportional hazards models were used in

the multivariable analysis with a proportionality assumption implicit in adjusted models. Relative hazard ratios were used to compare the risk of diabetes in the subjects with increased clinical periodontal measures (Category II-V) versus the group with minimal bleeding upon probing and shallow probing measurements (Category I). Relative hazard ratios were used to compare the risk of incident diabetes in the high versus low levels of serum antibody levels to the periodontal pathogens *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*) and high versus low levels of serum gingival crevicular fluid levels of IL-1 $\beta$  (GCF- IL-1 $\beta$ ) and prostaglandin (PG-E2). Missing data and participants positive for type 2 diabetes at baseline were excluded in each analysis. All tests of significance were two-tailed, with an  $\alpha$  level of 0.05. All analyses were performed using STATA 9.1 (Stata, College Station, TX).

## RESULTS

Baseline characteristics by clinical periodontal inflammation status (category I-V) were presented in **Table 14**. All the characteristics were considered statistically different between the groups if  $p < 0.05$ . The categories with more severe clinical inflammation were more likely to be female, smokers, and less physically active. These periodontal groups also display higher caloric intake, BMI, waist circumference, hypertension, HDL cholesterol, fasting glucose and 2 hr glucose tolerance levels. ( $P < 0.0001$ ) There were no differences in age ( $P = 0.158$ ), previous history of cardiovascular disease ( $P = 0.159$ ), and family history of diabetes ( $P = 0.071$ ). A subsidiary analysis of baseline characteristics of

4, 864 individuals available at visit 4, but excluded from the periodontal examination, was performed (results not shown). The individuals excluded from our primary analysis had a higher proportion of African-Americans (39% vs. 24%), smokers (19% vs. 13%), increased caloric intake (1773 kcal/day vs. 1587kcal/day) and higher body mass-indices (31kg/m<sup>2</sup> vs. 28.9kg/m<sup>2</sup> ).

Baseline characteristics by category of bleeding upon probing status were presented in **Table 15**. The characteristics were considered statistically different between the groups if  $p < 0.05$ . The categories with more severe bleeding upon probing ( $\geq 50\%$  of sites) were more likely to be female, smokers, and less physically active. These periodontal groups also display higher caloric intake, BMI, waist circumference, hypertension, HDL cholesterol, previous history of cardiovascular disease, family history of diabetes. fasting glucose and 2 hr glucose tolerance levels. ( $P < 0.0001$ ) There were no differences in age ( $P = 0.20$  ) among these three groups.

During 13.84 years of follow up, 1,967 individuals developed Type 2 diabetes of the total ( $n = 5,819$ ) participants. Missing data and participants positive for type 2 diabetes at baseline were excluded in each analysis. The incidence rate of diabetes with a healthy periodontal status was 17.4. per 1000 person-years (95%CI: 17.2-19.0), while the incidence rates for Category II and V clinical inflammation were significantly higher at 22.3 (95%CI: 20.9-23.7) and 23.9 (95%CI: 22.2-25.2) per 1000 person-years, respectively ( $p < 0.001$ ). (**Table**

**16)** The incidence of type 2 diabetes did not appear to increase monotonically across the 5 periodontal categories.

Kaplan-Meier analysis (**Figure 5**) and the associated log-rank tests, showed that the early and severe periodontal disease (Category II-(probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $>10\%$ , and Category V- one or more sites with PD $\geq 4$ mm, bleeding upon probing  $\geq 50\%$ ), had higher cumulative diabetes incidence ( $P < 0.0001$ ), than the reference group (Category I=probing depth (PD)  $\leq 3$ mm, bleeding upon probing  $\leq 10\%$ ), Category III (one or more sites with PD $\geq 4$ mm, bleeding upon probing  $\leq 10\%$ ) and Category IV (one or more sites with PD $\geq 4$ mm, bleeding upon probing  $>10\%$  and  $<50\%$ ) ( $p < 0.0001$ , **Figure 5**). No differences in incident diabetes could be seen in the KM-plots of antibody levels (high vs. low) to the pathogens *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*, and serum gingival crevicular fluid levels of IL-1 $\beta$  (GCF- IL-1 $\beta$ ) and prostaglandin (PG-E2) ( $P > 0.05$ , **Figures 6-9**).

In multivariable analyses, for the diabetes cases using the Cox proportional hazards model, the hazard ratio appeared the highest with early periodontal clinical measures of inflammation as found in Category II (1.4, 95%CI: 1.1-1.7  $p < 0.001$ ) after adjustment for sex, age, race, education level, smoking status, physical activity, total caloric intake, waist circumference, hypertension, previous cardiovascular disease, family history of diabetes, and HDL cholesterol levels. (**Table 16**) Compared with individuals with minimal bleeding and probing measures, the hazard of incident diabetes appears to be 1.2 times higher in adults with moderate (Category IV) (95%CI: 1.0-1.4,  $P < 0.001$ )

clinical periodontal inflammation and 1.3 times higher in adults with advanced periodontal inflammation (Category V) (95%CI: 1.0-1.6,  $P < 0.001$ ). However Category III did not display this increasing significant trend with a hazard ratio of 1.0 (95% CI: 0.8-1.20). As expected, additional adjustment including fasting glucose (model 6) or 2-hr GTT (model 7) further attenuated the association, because they were both in the causal pathway. A dose-response relationship with clinical inflammation could be seen by using only bleeding upon probing as a measure for clinical inflammation. (**Table 17**). Censoring of individuals who died during follow-up ( $n = 211$ ) also did not change the associations observed with incident diabetes (data not shown).

To investigate the relationship of incident diabetes to other inflammatory measures of periodontal disease exposure, additional analyses including participants with assays of periodontal inflammation were performed. First, to determine if systemic markers specific to exposure to periodontal inflammation might help explain the relationship of periodontal disease to diabetes risk, antibodies to the periodontal pathogens *Porphyromonas gingivalis* and *Actinobacillus actinmycetemcomitans* were included into multivariable models adjusted for age, sex, race, smoking, waist circumference, cardiovascular disease, family history of diabetes, total caloric intake, and cholesterol levels. The hazard of incident diabetes appeared no different in adults with high levels of antibodies to *Porphyromonas gingivalis* compared to low serum levels. The hazard ratios for antibodies to *Actinobacillus actinmycetemcomitans* also



appeared to be in these ranges but did not reach statistical significance. (**Table 18**)

Data on localized markers for periodontal inflammation were available, and additional adjusted multivariable analyses using gingival crevicular fluid were performed. High levels of gingival crevicular fluid IL-1 $\beta$  were associated with no change in hazard of incident diabetes (HR=1.0, CI:0.8-1.2). High levels of 1 $\beta$  PG-E2 were also associated with no increased hazard of incident diabetes (1.0: 95% CI: 0.8-1.1). (**Table 18**)

**Table 14- Baseline Characteristics of 5, 819 Visit 4 participants receiving dental examinations excluding diagnosed and undiagnosed subjects with diabetes-ARIC Dental Study (1996-1998 to present).**

	Category I	Category II	Category III	Category IV	Category V	P value
N	860	861	1,084	2,326	688	
Male sex (%)	73.4	64.2	53.0	50.8	40.3	<0.0001
Age (years)	62.2±5.5	62.3±5.8	62.4±5.6	62.3±5.6	62.7±5.8	0.198
African American (%)	28.4	23.0	9.5	10.1	29.5	<0.0001
Education ≤12 Years (%)	53.9	48.3	63.6	52.3	44.9	<0.0001
Smoking Status (%) - Current	10.6±0.3	8.6±0.3	14.4±0.4	12.4±0.3	18.1±0.4	<0.0001
Sports Index	2.5±0.8	2.5±0.8	2.7±0.8	2.6±0.8	2.4±0.8	<0.0001
Total Calorie Intake (Kcal/day)	1548±668	1556±595	1564±574	1627±634	1739±786	<0.0001
Body mass index (kg/m <sup>2</sup> )	28.3±5.6	28.5±4.55	27.5±4.5	28.4±5.0	29.0±5.5	<0.0001
Waist Circumference (cm)	101.9±14.6	104.5±14.3	102.6±12.7	103.8±13.5	107±13.9	<0.0001
Hypertension (%)	45.5	42.5	32.0	38.9	48.6	P<0.0001
Previous Cardiovascular Disease (%)	4.5	4.5	5.8	5.3	7.0	0.1590
Family History of Diabetes (%)	12.2	13.4	12.1	10.8	13.2	0.0710
High Density Lipoprotein (mmol/L)	1.4±0.4	1.6±0.4	1.3±0.4	1.4±0.4	1.7±0.4	<0.0001
Mean Fasting Glucose (mg/dL)	98.1±9.4	99.0±9.6	97.8±9.1	99.0±9.2	101.2±9.2	<0.0001
Mean 2hr Glucose Tolerance Test (mg/dL)	131.0±39	134.4±40	123.0±40	130.3±41	132.3±40	<0.0001

Characteristics were statistically different if  $p < 0.05$  using ANOVA tests for continuous variables and  $\chi^2$  for categorical variables

Category I=probing depth (PD) ≤3mm, bleeding upon probing ≤10% (reference category), Category II=probing depth (PD) ≤3mm, bleeding upon probing >10%, Category III=one or more sites with PD≥4mm, bleeding upon probing ≤10%, Category IV=one or more sites with PD≥4mm, bleeding upon probing >10% and <50%, Category V=one or more sites with PD≥4mm, bleeding upon probing ≥50%

**Table 15-Baseline Characteristics of 5, 819 Visit 4 participants receiving dental examinations excluding subjects with diagnosed and undiagnosed diabetes**

	Mild BOP	Moderate BOP	Severe BOP	P value
N	1,944	3,073	802	
Male sex (%)	62.0	54.7	41.4	<0.0001
Age (years)	62.3±5.5	62.3±5.6	62.8±5.8	0.1979
African American (%)	17.9	12.9	29.8	<0.0001
Education ≤12 Years (%)	53.9	48.3	63.6	<0.0001
Smoking Status (%) -Current	12.7	11.2	17.8	<0.0001
Sports Index	2.6±0.8	2.5±0.8	2.4±0.8	<0.0001
Total Calorie Intake (Kcal/day)	1556±617	1596±620	1731±776	<0.0001
Body mass index (kg/m <sup>2</sup> )	27.7±5.0	28.2±5.1	29.0±5.6	<0.0001
Waist Circumference (cm)	98.2±13.6	100.4±13.7	102.7±14.1	<0.0001
Hypertension (%)	38.0	39.5	48.8	<0.0001
Previous Cardiovascular Disease (%)	5.2	5.0	6.8	<0.0001
Family History of Diabetes (%)	12	12	13	<0.0001
High Density Lipoprotein (mmol/L)	1.4±0.4	1.3±0.4	1.3±0.4	<0.0001
Total Calorie Intake (Kcal/day)	1556±617	1596±620	1731±776	<0.0001
Mean Fasting Glucose (mg/dL)	98.0±9.2	98.9±9.3	101.3±9.4	<0.0001
Mean 2hr Glucose Tolerance Test (mg/dL)	126.5±40.1	131.1±41.0	133.2±41.1	<0.0001

Characteristics were statistically different if  $p < 0.05$ , using ANOVA tests for continuous variables and  $\chi^2$  for categorical variables

Mild BOP= bleeding upon probing ≤10% (reference category)

Moderate BOP= bleeding upon probing 10-49%

Severe BOP= bleeding upon probing ≥50%

**Table 16- Relative Hazard of Type 2 Diabetes over 13.8 years follow-up by Category of Clinical Periodontal Inflammation**

	Category 1	Category 2	Category 3	Category 4	Category 5
Incidence rate (per 1000 person years)	17.4 (17.2-19.0)	22.3 (20.9-23.7)	13.6 (12.5-14.1)	17.8 (16.2-18.1)	23.9 (22.2-25.2)
Model 1	1.0	1.3 (1.0-1.6)	0.8 (0.6-1.0)	1.1 (0.9-1.3)	1.4 (1.1-1.8)
Model 2	1.0	1.3 (1.1-1.6)	0.9 (0.7-1.1)	1.1 (0.9-1.4)	1.4 (1.1-1.7)
Model 3	1.0	1.3 (1.1-1.6)	0.9 (0.7-1.1)	1.1 (0.9-1.3)	1.3 (1.1-1.7)
Model 4	1.0	1.3 (1.0-1.6)	0.9 (0.7-1.1)	1.1 (0.9-1.3)	1.2 (1.0-1.6)
Model 5	1.0	1.4 (1.1-1.7)	1.0 (0.8-1.3)	1.2 (1.0-1.4)	1.3 (1.0-1.6)
Model 6	1.0	1.4 (1.1-1.8)	1.1 (0.9-1.5)	1.3 (1.0-1.6)	1.3 (1.0-1.7)
Model 7	1.0	1.3 (1.0-1.7)	1.0 (0.8-1.3)	1.2 (1.0-1.4)	1.1 (0.9-1.5)

Model 1- unadjusted

Model 2- adjusted for sex, age, race, and education

Model 3- adjusted for lifestyle covariates (sex, age, race, education, physical activity, smoking total caloric intake)

Model 4-adjusted for lifestyle covariates and waist circumference

Model 5- fully (for sex, age race, education, smoking, total caloric intake, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol)

Model 6- fully adjusted (for sex, age race, education, smoking, total caloric intake, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol) plus 2-hour glucose tolerance test level (continuous variable)

Model 7- fully adjusted (for sex, age race, education, smoking, total caloric intake, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol) plus fasting glucose (continuous variable)

Category I=probing depth (PD) ≤3mm, bleeding upon probing ≤10% (reference category)

Category II=probing depth (PD) ≤3mm, bleeding upon probing >10%

Category III=one or more sites with PD≥4mm, bleeding upon probing ≤10%

Category IV=one or more sites with PD≥4mm, bleeding upon probing >10% and <50%

Category V=one or more sites with PD≥4mm, bleeding upon probing ≥50%

**Table 17-Relative Hazard of Type 2 Diabetes over 13.7 years follow-up by Category of Clinical Bleeding Upon Probing**

	Mild BOP	Moderate BOP	Severe BOP
Incidence rate (per 1000 person year)	15.2 (14.1-16.3)	18.7 (16.0-19.2)	24.8 (22.9-26.4)
Model 1	1.0	1.2 (1.2-1.7)	1.7 (1.4-2.0)
Model 2	1.0	1.2 (1.1-1.4)	1.5 (1.3-1.8)
Model 3	1.0	1.2 (1.1-1.4)	1.5 (1.2-1.8)
Model 4	1.0	1.2 (1.0-1.4)	1.4 (1.1-1.6)
Model 5	1.0	1.2 (1.1-1.4)	1.3 (1.0-1.5)
Model 6	1.0	1.3 (1.1-1.5)	1.2 (1.0-1.5)
Model 7	1.0	1.2 (1.0-1.4)	1.1 (0.9-1.4)

Model 1- unadjusted

Model 2- adjusted for sex, age, race, and education

Model 3- adjusted for lifestyle covariates –(sex, age, race, education, physical activity, smoking total caloric intake)

Model 4-adjusted for lifestyle covariates and waist circumference

Model 5- fully adjusted (for sex, age race, education, smoking, total caloric intake, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol)

Model 6- fully adjusted (for sex, age race, education, smoking, total caloric intake, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol) plus 2-hour glucose tolerance test level (continuous variable)

Model 7- fully adjusted (for sex, age race, education, smoking, total caloric intake, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol) plus fasting glucose (continuous variable)

Mild BOP= bleeding upon probing  $\leq 10\%$  (reference category)

Moderate BOP= bleeding upon probing 10-49%

Severe BOP= bleeding upon probing  $\geq 50\%$

**Table 18- Relative Hazard of Type 2 Diabetes over 13.7 years follow-up by Systemic Markers for Periodontal Inflammation**

	Antibody to P. gingivalis (high vs. low)	Antibody to A.a. (high vs. low)	GCF levels of IL-1 $\beta$ (high vs. low)	GCF levels of PG-E2 (high vs. low)
Incidence rate(per 1000person year)	0.058 (-0.02-0.42)	0.057 (-0.02-0.75)	0.058 (0.006-0.091)	0.060 (-0.0018-0.13)
Model 1	1.1 (1.0-1.3)	1.1 (1.0-1.3)	1.0 (0.9-1.1)	1.0 (0.9-1.1)
Model 2	1.1 (0.9-1.2)	1.0 (0.9-1.2)	1.0 (0.9-1.2)	1.0 (0.9-1.1)
Model 3	1.1 (0.9-1.2)	1.0 (0.9-1.2)	1.0 (0.9-1.2)	1.0 (0.9-1.1)
Model 4	1.1 (0.9-1.2)	1.1 (1.0-1.2)	1.0 (0.9-1.1)	1.0 (0.9-1.1)
Model 5	1.0 (0.9-1.2)	1.0 (0.9-1.2)	1.0 (0.9-1.2)	1.0 (0.8-1.1)
Model 6	1.0 (0.9-1.2)	1.0 (0.8-1.2)	1.0 (0.8-1.2)	1.0 (0.8-1.2)
Model 7	1.0 (0.8-1.2)	1.1 (0.9-1.3)	1.1 (1.9-1.3)	1.0 (0.8-1.2)

Model 1- unadjusted

Model 2- adjusted for sex, age, race, and education

Model 3- adjusted for lifestyle covariates –(sex, age, race, education, physical activity, smoking, total caloric intake)

Model 4-adjusted for lifestyle covariates and waist circumference

Model 5- fully adjusted (for sex, age race, education, smoking, total caloric intake, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol)

Model 6- fully adjusted (for sex, age race, education, smoking, total caloric intake, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol) plus 2-hour glucose tolerance test level (continuous variable)

Model 7- fully adjusted (for sex, age race, education, smoking, total caloric intake, waist circumference, sports index, CHD, family history diabetes, hypertension, cholesterol) plus fasting glucose (continuous variable)

High *Porphyromonas gingivalis* antibody levels at  $\geq 78.93$  EU (highest quartile)

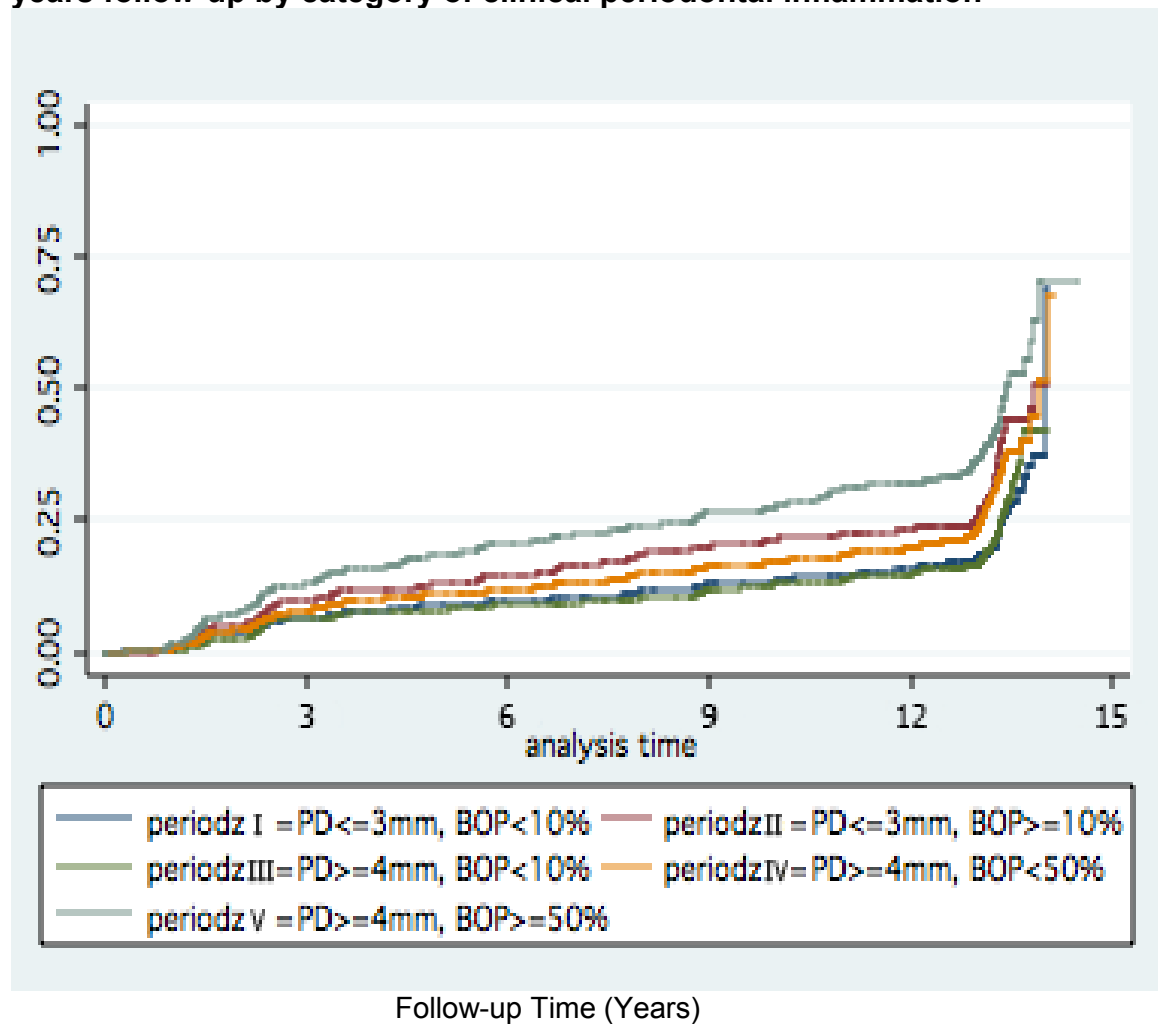
High *Actinobacillus actinomycetemcomitans* antibody levels  $\geq 144$  EU (highest quartile)

High GCF- IL-1 $\beta$  levels at  $\geq 146$ ng/mL (highest quartile)

High GCF PG-E2 levels  $\geq 239$ ng/mL (highest quartile)

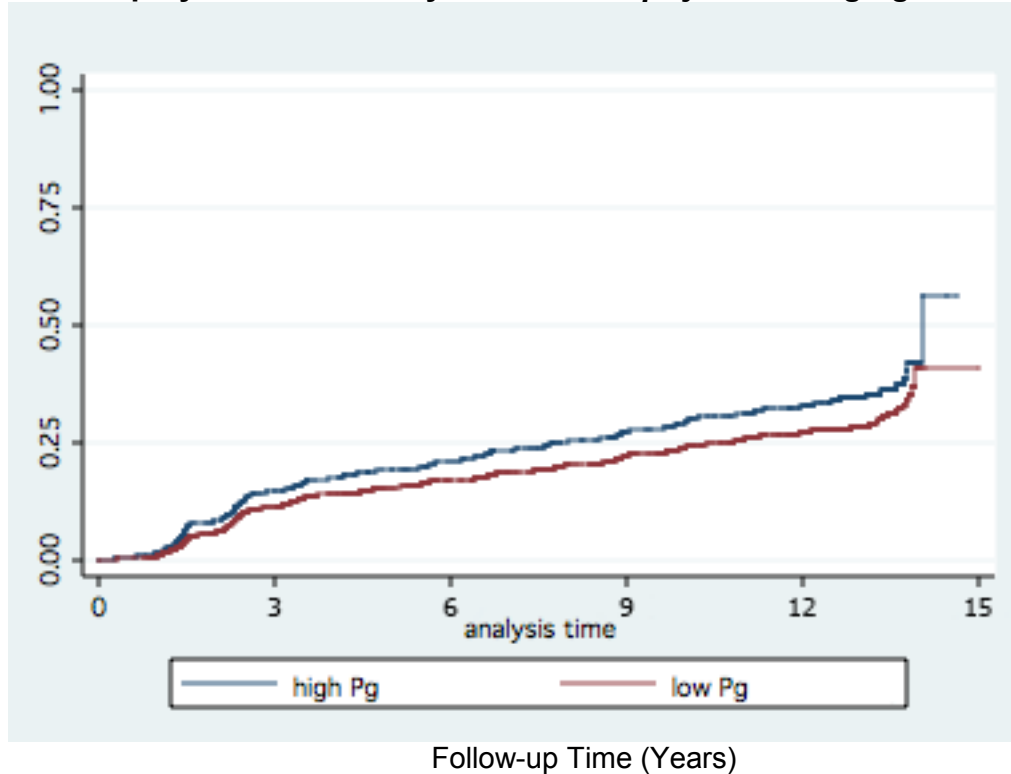
Low= lower 3 quartiles for all markers

**Figure 4: - Cumulative probability of incident type 2 diabetes over 13.8 years follow-up by category of clinical periodontal inflammation**



Log-rank test  $p < 0.001$

**Figure 6: Cumulative probability of incident type 2 diabetes over 13.8 years follow-up by serum antibody levels to *Porphyromonas gingivalis***



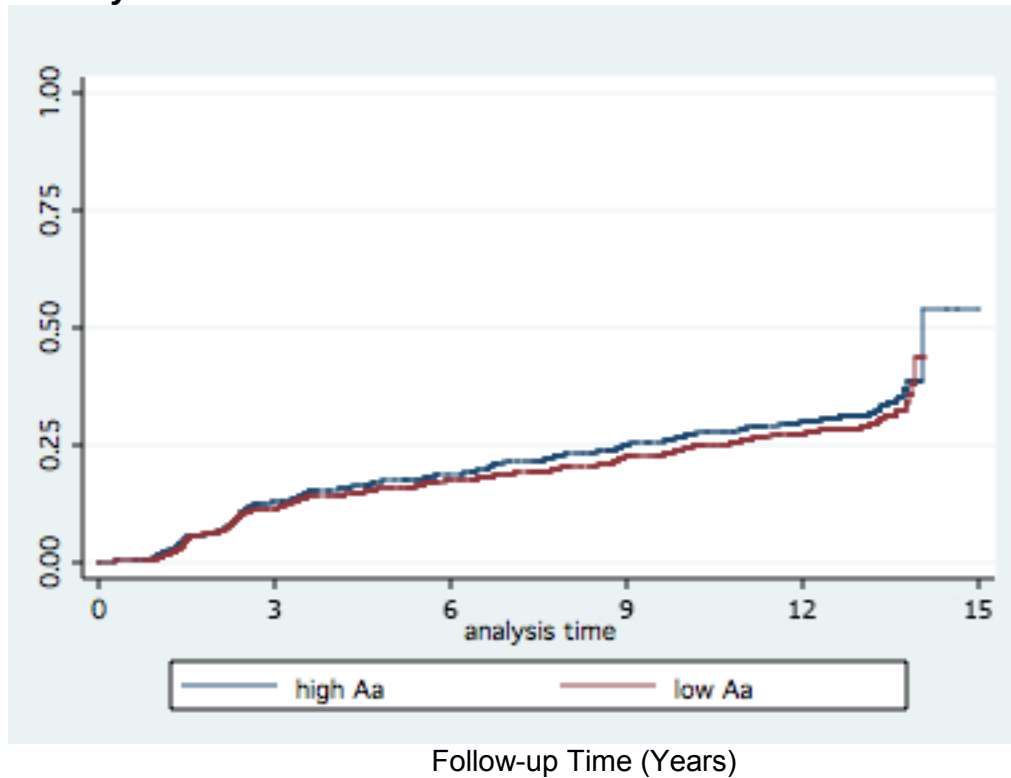
Log-rank test  $p > 0.05$

High *Porphyromonas gingivalis* antibody levels at  $\geq 78.93$  EU (highest quartile)

Low *Porphyromonas gingivalis* antibody levels at  $< 78.93$  EU (lower three quartiles)



**Figure 7: Cumulative probability of incident type 2 diabetes over 13.8 years follow-up by serum antibody levels to *Actinobacillus actinmycetemcommitans***

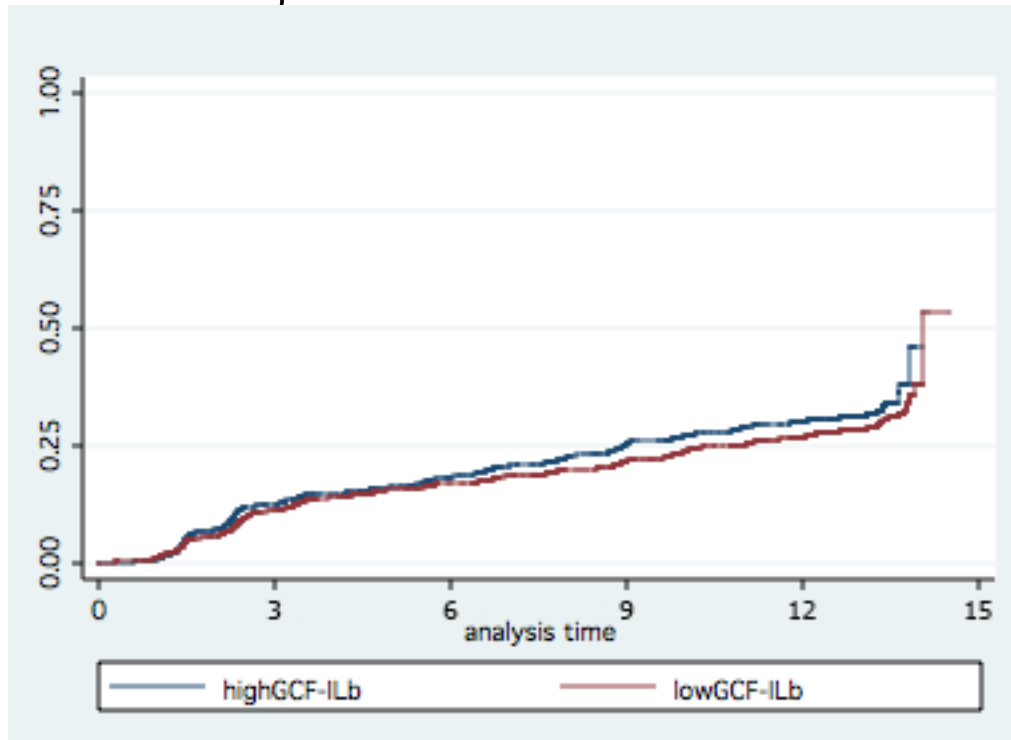


Log-rank test  $p > 0.05$

High *Actinobacillus actinmycetemcommitans* antibody levels  $\geq 144$  EU (highest quartile)

Low *Actinobacillus actinmycetemcommitans* antibody levels  $< 144$  EU (lower three quartiles)

**Figure 8: Cumulative probability of incident type 2 diabetes over 13.8 years follow-up by GCF levels of IL-1 $\beta$**



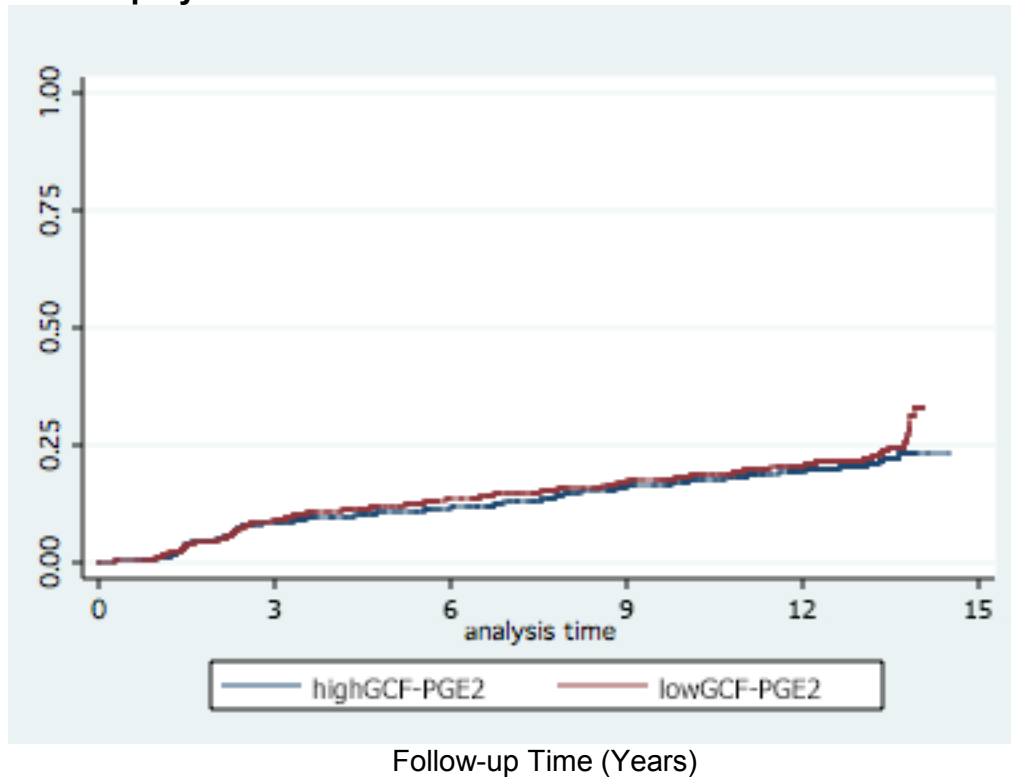
Follow-up Time (Years)

Log-rank test  $p > 0.05$

High GCF- IL-1 $\beta$  levels at  $\geq 146$ ng/mL (highest quartile)

Low GCF- IL-1 $\beta$  levels at  $< 146$ ng/mL (lower three quartiles)

**Figure 9: Cumulative probability of incident type 2 diabetes over 13.8 years follow-up by GCF levels of PG-E2**



Log-rank test  $p > 0.05$

High GCF PG-E2 levels  $\geq 239$  ng/mL (highest quartile)

Low GCF PG-E2 levels  $< 239$  ng/mL (lower three quartiles)

## Discussion

In a longitudinal analysis of this cohort, clinical parameters of periodontal inflammation at baseline increased the risk of incident diabetes over a 13.84 year follow-up. As observed in the cross-sectional design (Chapter 2), serum markers for inflammation were not associated strongly with incident diabetes. No association with incident diabetes was seen with high baseline levels of IgG antibody levels to *Porphyromonas gingivalis*, and *Actinobacillus actinomycetemcomitans*, and this lack of association remained consistent when analyzing gingival crevicular fluid IL- $\beta$  and PG-E2 levels.

Another study found no association of clinical periodontal disease with incident diabetes in Japan. This study used fasting glucose levels similar to our study with a similar sample size (n=5,848), but the study duration was only 7 years, which may not be sufficiently long enough to observe incident cases.<sup>57</sup>

Only one other study has found a positive association of baseline clinical periodontal disease and risk of subsequent diabetes.<sup>56</sup> In the National Health and Nutrition Examination Survey (NHANES) including 7,168 eligible participants, after 17 years of follow-up, the odds ratios for incident diabetes ranged from 1.5 (95% CI: 0.99-2.27) in advanced periodontal disease to 2.26 (95%CI: 1.56-3.27) in moderate periodontitis. That study used the periodontal index to classify severity of periodontal inflammation, which looked at the visual

extent of gingival inflammation, presence or absence of pockets and tooth mobility to assign an averaged score. Our study used a comprehensive examination of probing measurements and bleeding upon probing, which are both the standard of care in clinical practice for diagnosing periodontal disease. The NHANES study also used death certificates, self-reports of diabetes requirement of pharmacologic treatment, and a health care facility stay with a discharge code of diabetes, which may have overestimated the number of new cases. Those participants were followed up at least one time. Our study was strengthened by yearly follow-up telephone calls, which was more likely to identify true incident diabetes as they occurred.

Our results did not support the findings found in CVD outcome studies where systemic markers for periodontal inflammation were associated with an increased risk of cardiovascular disease. Both high antibody levels, *Porphyromonas gingivalis* and *Actinobacillus actinmycetemcomitans*, have been found to increase the risk of CVD by an overall odds ratio of 1.75 (95%CI: 1.32 to 2.34)<sup>54</sup> No studies have assessed local inflammatory markers such as gingival crevicular fluid IL- $\beta$  and PG-E2 with cardiovascular or diabetes risks. Periodontal treatment for advanced periodontitis has not been shown to reduce inflammatory mediators in diabetic subjects, though A1C levels were significantly improved.<sup>55</sup>

Our study had several strengths. First, ARIC is a large, community-based, biracial population in which there was standardized ascertainment of follow-up for approximately 14 years. Second, there were standardized measures of exposures, outcomes, and confounding variables in a rigorously monitored observational study, allowing us to explore the associated risk of incident diabetes with prior periodontal disease exposures. This study is novel by combining both clinical and systemic measures specific to periodontal inflammation to assess diabetes as an outcome. This approach has been used to assess cardiovascular disease as an outcome, but unlike these other cardiovascular studies, an association of systemic inflammatory mediators with increased risk of diabetes was not shown.<sup>50, 51</sup> Our study suggests that the association of periodontal inflammation with risk of diabetes is not the same as the association with the risk of cardiovascular disease. In the cardiovascular disease infection hypothesis, several studies have validated the use of serum antibody level to the periodontal pathogens *Porphyromonas gingivalis* and *Actinobacillus actinmycetemcomitans* as a surrogate of periodontal clinic exam when assessing CVD risk.<sup>51, 54</sup> While these serum antibody levels do not indicate active or current periodontal disease, they have been used to study the level of prior exposure to periodontal inflammation and CVD risk.

Nonetheless, the limitations should be kept in mind when interpreting our data. Firstly, this study also lacked longitudinal dental and medical exams. Teeth and their surrounding tissues provide the niche for periodontal pathogens and

gingival crevicular fluid. One study found that the elevated serologies no longer conferred increased cardiovascular risk in edentulous subjects.<sup>51</sup> Tooth loss data was not available after the baseline visit and dietary data was scant in this dataset. Tooth loss may also influence dietary choices, caloric intake, cholesterol levels, body mass index and diabetes. While these were included in the model as confounders, tooth loss and diet may be a distinct separate pathway in the direction from periodontal disease to diabetes. The longitudinal NHANES study found that participants with no teeth, had an odds ratio for incident diabetes of 1.3 (95%CI: 1.0-1.7), and those with advanced tooth loss (1-7 teeth remaining) had an odds ratio of 1.7 ( $P<0.05$ ).<sup>56</sup> Blood glucose assessment was also not available at follow-up to confirm incident diabetes in our study to confirm the telephone questionnaire responses.

A subsidiary analysis of baseline characteristics of 4,864 individuals available at visit 4, but excluded from the periodontal examination, was performed (results not shown). The individuals excluded from our primary analysis had a higher proportion of African-Americans (39% vs. 24%), smokers (19% vs. 13%), increased caloric intake (1773 kcal/day vs. 1587kcal/day) and higher body mass-indices ( $31\text{kg/m}^2$  vs.  $28.9\text{kg/m}^2$ ). These aforementioned characteristics are known risk factors for diabetes. It is possible that exclusion of these individuals from the analysis may have resulted in an underestimated risk of diabetes. Additionally, 15 % ( $n=1,478$ ) of the visit 4 participants were edentulous. If we assume that tooth loss is a surrogate for severe periodontal

disease status, then it is possible a large proportion of individuals with prior exposure to severe periodontal inflammation were not available for analysis, also resulting in an underestimated risk of diabetes.

We used the serum levels for inflammatory mediators' highest quartile as the cut-point for high vs. low levels. Other studies used the highest tertile or quartile for the high level category for studying the association of periodontal disease with cardiovascular disease risk.<sup>51</sup> It is possible that the highest tertile or quartile may represent a unique population with the possibility for residual confounding.

Performing multiple regressions for the five clinical and four systemic markers of inflammation increased the possibility of Type I error. The possibility of a false positive merely due to chance may also be due to the large number of models produced for dividing diabetes diagnosis into several categories.

## **Conclusions**

The Atherosclerosis Risk in Communities (ARIC) Study is a community-based prospective cohort providing a rich database with which to assess the effect of periodontal disease exposure on incident diabetes. This study helps answer the recent call by the Joint EFP/AAP consensus report for studies with



comprehensive clinical data, extent and severity of periodontal disease, level of glycemic control, and consideration of local and systemic pathways affected both periodontal disease and diabetes.<sup>53</sup> This study served to contribute to the body of evidence that is largely lacking in the directionality of periodontal disease and subsequent incident diabetes.

This study supports the hypothesis that clinical periodontal inflammation increases the risk of incident diabetes several years later. An increase in bleeding upon probing appears to be both cross-sectionally associated with impaired glucose tolerance and longitudinally associated with the onset of incident diabetes. Since even minimal bleeding upon probing in this study was associated with prevalent pre-diabetes and future incident diabetes, a strong case is made to support patient education for prevention of periodontal disease to and study the effect of prevention of even mild periodontal inflammation on impaired glucose tolerance and diabetes.

The serum markers specific to periodontal disease used in cardiovascular disease models do not appear to be helpful in assessing risk of incident diabetes. Though the American Heart Association's Scientific Statement on Diabetes stated that "diabetes is a cardiovascular disease", the mechanism of action may be very different.<sup>58</sup> It may not be enough to study just a few systemic and local markers for periodontal disease to understand the mechanistic pathway of periodontal diseases and increased risk of type 2 diabetes.

## **CHAPTER 4**

### **Periodontists' attitudes, beliefs and standard of care in treating dental patients at risk for diabetes: A survey in Washington DC area**

#### **Abstract**

The two-way relationship of periodontal disease and diabetes has been discussed in the literature for almost two decades, while the evidence to support the risk of diabetes associated with periodontal disease exposure is sparse. The association of periodontitis with type 2 diabetes is recognized by local Periodontists (using a convenience sample survey of Washington DC area Periodontists), and the attitudes and beliefs of these specialists influence the standard of care in treating dental patients. When asked if it was appropriate to probe further about of diabetes risk factors in patients with periodontal disease and no diabetes diagnosis, most respondents (92.9%) agreed (agreed/ strongly agreed, n=39). This survey suggests that practicing periodontists are aware that an association between periodontal disease and onset of type 2 diabetes, and

they appear aware of the importance of HbA1c testing in assessing glycemic control, whether this test is performed in the dental office or medical setting. This appears to parallel the consensus report of the Joint European Federation/ American Academy Workshop (EFP/AAP) guidelines to dentists for patients without a diabetes diagnosis, but obvious risk factors for type 2 diabetes.

## **Introduction**

Periodontal disease is the most common inflammatory condition worldwide and diabetes is quickly becoming a global epidemic. The bidirectional pathway of periodontal disease and diabetes is not fully understood. While consistent evidence has shown that diabetes is related to periodontitis, emerging evidence suggests that periodontal disease may increase the risk of diabetes onset.

Risk factors for type 2 diabetes include older age, obesity, and family history of diabetes, hypertension, high cholesterol levels and history of vascular disease.<sup>5</sup> Modifiable lifestyle risk factors include smoking, physical activity level, weight loss, and healthy diet.<sup>5</sup> While periodontal disease as a risk factor for incident diabetes has been proposed, sufficient evidence to quantify this association is lacking.<sup>59</sup>

The consensus report of the Joint European Federation/ American Academy Workshop (EFP/AAP) on periodontitis and systemic disease recently reviewed the role of periodontitis and the associated the risk of type 2 diabetes.

Their guidelines to dentists for patients without a diabetes diagnosis, but obvious risk factors for type 2 diabetes, include that the patients:

“should be informed of their risk for having diabetes, assessed using a chair-side HbA1C test, and/or referred to a physician for appropriate testing and diagnostic care.”<sup>53</sup>

This joint EFO/AAP suggests that evidence is emerging about the role of periodontal inflammation and the risk of incident diabetes, but concluded, “there is lack of clarity in the literature regarding the strength of this latter association”.<sup>53</sup> This joint consensus report concluded that because of the “relative immaturity of the body of evidence for this purported relationship, the field is wide open and the gaps in knowledge are large”.<sup>53</sup> Therefore, we conducted this survey to better understand the beliefs, perceptions, and current practices among local Periodontists in treating periodontal patients who may be at risk for diabetes.

**Hypothesis:**

The association of periodontitis with diabetes with type 2 diabetes is recognized by Periodontists , and the attitudes and beliefs of these specialists influence the standard of care in treating dental patients.

**Methods*****Identification of Potential Study Population***

We identified potential participants by examining the Periodontist listed by the American Academy Periodontology (AAP) as active members of the AAP. Additionally, only those listed within a 50-mile radius of Howard University were contacted. These periodontists self-selected for inclusion by choosing to participate in the survey. The institutional review board of Howard University approved this study with a waiver for informed consent.

***Survey Content***

The survey consisted of 6 questions. Three questions were rated on a four-point Likert-type scale and asked Periodontists about their practices in

treating patients who have not yet been diagnosed with type 2 diabetes. Two more survey items were unique questions that were asked based on respondent's previous answers (using skip logic functions), and assessed the beliefs of the providers for practice decisions (ranking answers, and multiple answers). The final question was open-ended, which asked information about the number of years the respondents have been practicing.

### ***Survey Process***

A confidential, self-administered survey instrument was developed with consultation with experts in survey design and methodology. Specialists in the field of dentistry assisted with the content and pre-tested the survey tool. The survey was modified to reflect changes suggested from these reviewers, and then it was transferred to an electronic format using a web-based survey service ([www.surveymonkey.com](http://www.surveymonkey.com)). The electronic and written versions of the survey were then pilot tested by having reviewers complete the survey. Based on our pilot testing, the survey took between 3-5 minutes to complete, regardless whether the survey was done on paper or via the web-based format.

An e-mail invitation with an imbedded html link to the web-survey was sent to all 100 participants who agreed to take the survey, with two subsequent reminder e-mails sent at five days and ten days to non-responders, and a second telephone call at 7 days to this group. The invitation included an endorsement

from the Dean of Howard University College of Dentistry, who was also the interim Deputy Provost of Health Sciences at Howard University. The invitation to participate in the survey was initiated in the middle of February 2014, and all responses to the middle of March 2014 were included for analysis. **(See Appendix -Survey Questions from Chapter 4, p.120-120)**

### ***Measurement and Data Analysis***

All survey data were downloaded from the web-based application. Data management and analysis were completed using STATA 9.1 (Stata, College Station, TX). Descriptive statistics for all data was reported, using means and percents. Questions about the likelihood of discussing risk factors and comfort level of discussing these risk factors were dichotomized (very unlikely/unlikely and very likely/likely), as were questions about appropriateness of discussing risk factors (strongly disagree/disagree and strongly agree/agree) and level of comfort in screening for diabetes (very comfortable/comfortable and very uncomfortable /uncomfortable), Questions requiring ranking of answers (most important=1, least important=5, or N/A) were given an average rating of importance from a scale of 1-5.

## **Results**

### ***Study Participants***

Of 146 members Periodontists listed by the American Academy of Periodontology within a 50-mile radius of Howard University, 12 were not practicing in the area (retired, moved, or on medical leave), and 28 were duplicate listings (same provider at multiple office addresses). This left 106 periodontists eligible to participate in the survey, of which 6 declined during the first telephone call. The remaining 100 Periodontists agreed to participate and were sent web-based surveys. All 100 recipients chose the web-based format over telephone or paper responses. Of the 100 survey recipients, 42 initiated the survey and 39 completed the entire survey. The number of years in specialty practice ranged from 2 to 50 years (mean of 23.0 yrs, standard deviation SD=12.2 yrs).

### ***Risk Factors for Diabetes***

Periodontists were asked how likely they were to discuss risk factors for diabetes such as family history of diabetes, smoking, diet, exercise, and cardiovascular disease, in their periodontal patients who have not been diagnosed with diabetes. When asking about likelihood of discussing these risk factors 31.7% (n=13) were unlikely (very unlikely/ somewhat unlikely) and 68.3



(n=28) were likely (somewhat likely/ very likely) to discuss these risk factors. (Figure 9 and Table 19) When asked if it was appropriate to probe further about these risk factors in these same patients (periodontal disease and no diabetes diagnosis), 7.1% disagreed (strongly disagrees/ disagreed, n=3), and 92.9% agreed (agreed/ strongly agreed, n=39). (**Figure 11, Table 20**)

Those who disagreed (n=2) in the second question provided reasons for not probing further about risk factors for diabetes in their patients. Neither responder cited inadequate time during the dental visit as an important reason for not discussing risk factors for diabetes, but did feel, in the order of most important to least important: this is a discussion best addressed by the primary care physician (average rating= 2.5/5), the patient would not expect the Periodontist to do this (average rating= 3/5), there is not enough evidence about the risk factors for diabetes (average rating= 4/5), there is not enough evidence to suggest that periodontal disease increases the risk of diabetes(average rating= 5/5) , and they were not comfortable discussing these risk factors (average rating= 5/5).

Those who agreed in the second question (n=37), skipped to a question regarding the reasons for probing further about risk factors for diabetes in their patients. (**Figure 12**) In this discussion of risk factors of diabetes with patients, most felt; 1) This is an important teaching moment for the patient (89%, N=3), 2) There is sufficient evidence to suggest that periodontal disease increases the risk

of diabetes (81%, N=30), 3) This is a discussion best addressed by both the periodontist and the primary care physician (81%, N=30), 4) There is sufficient evidence about the risk factors for diabetes (76%, N=28), 5) There is adequate time during the appointment to have this discussion (70%, N=26), and 6) Feel comfortable discussing these risk factors (65%, N=24). They felt, in order of most important to least important: There is sufficient evidence about the risk factors for diabetes (average rating 2.5/5), there is sufficient evidence to suggest the periodontal disease increases the risk of diabetes (average rating= 2.75/5), they were comfortable discussing these risk factors (average rating= 3/5), this is an important teaching moment for the patient that should not be bypassed (average rating=3.3/5), this is a discussion best addressed by both the periodontist and the primary care physician (average rating=3.8/5), and there is adequate time during the appointment to have this discussion (average rating= 4.7/5). (**Table 21**)

### ***Screening for Type 2 Diabetes in the Dental Setting***

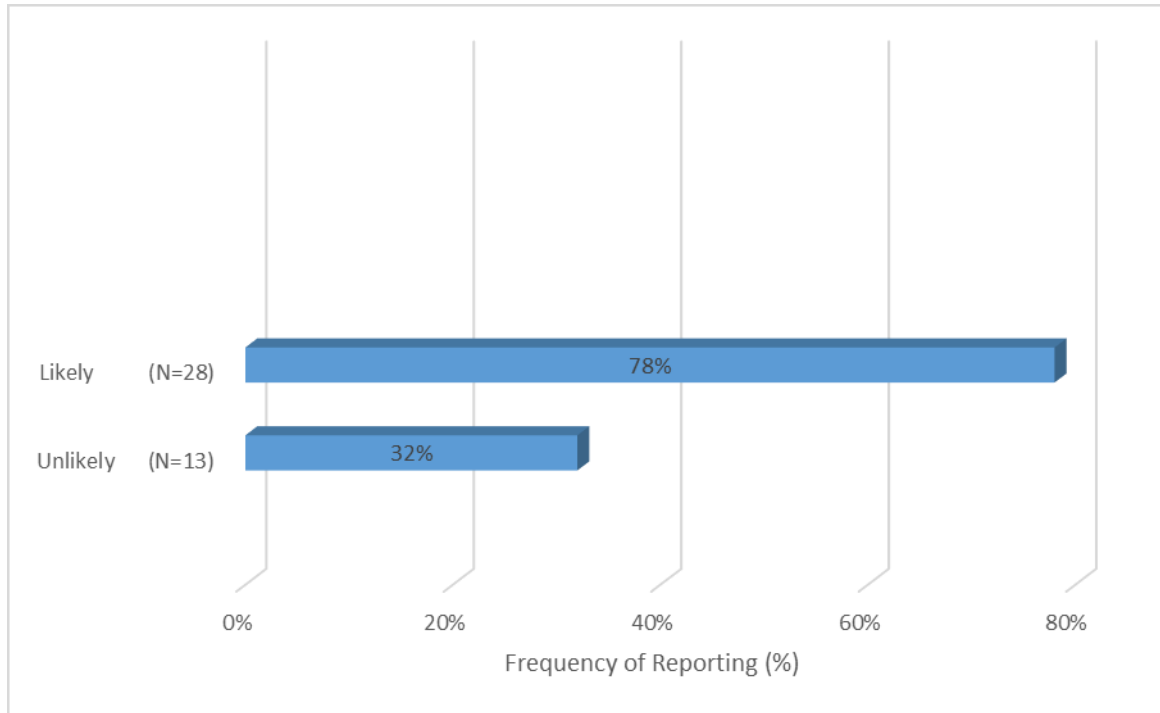
All survey respondents (n=39) were asked if they were comfortable performing a chair-side HbA1c test for assessing glycemic control, and 54.95% (n=20) felt uncomfortable, while 48. 7% (n= 19) were comfortable performing an HbA1c test. (**Figure 13**). Those uncomfortable in performing an in-office HbA1c test were asked the reasons for their discomfort, and 80% (n=16) felt that the physicians office was better equipped to perform such a test, 30% (n=6) did not feel comfortable performing this test in their office, 20% (n=4) did not feel they could be adequately reimbursed, 20% (n=4) said it was not a current standard of

care in dentistry, and 5% (n=1) reported that the effect of periodontal disease influencing HbA1c levels is not fully understood. **(Figure 14)**

Those comfortable in performing an in-office HbA1c test were asked the reasons for their comfort level and 76.5% (n=13) reported that they felt comfortable performing the test in the periodontal practice, 53% (n=9) reported that the effect of periodontal disease influencing HbA1c levels is well understood, 29.4% (n=5) felt the periodontal office is equipped to perform such a test, and 5.9% (n=1) said that it is a current recommended standard of care in dentistry. This group did not report a concern for being reimbursed for this procedure (0%, n=0). **(Figure 15)**

Of those uncomfortable in performing the HbA1c test in the dental office, 80% (N=16) felt that the physician's office is better equipped to perform such as test. **(Figure 14)** Over 50% of those comfortable in performing this test for glycemic control in the dental office felt that the effect of periodontitis on HbA1c is well understood. **(Figure 15)**

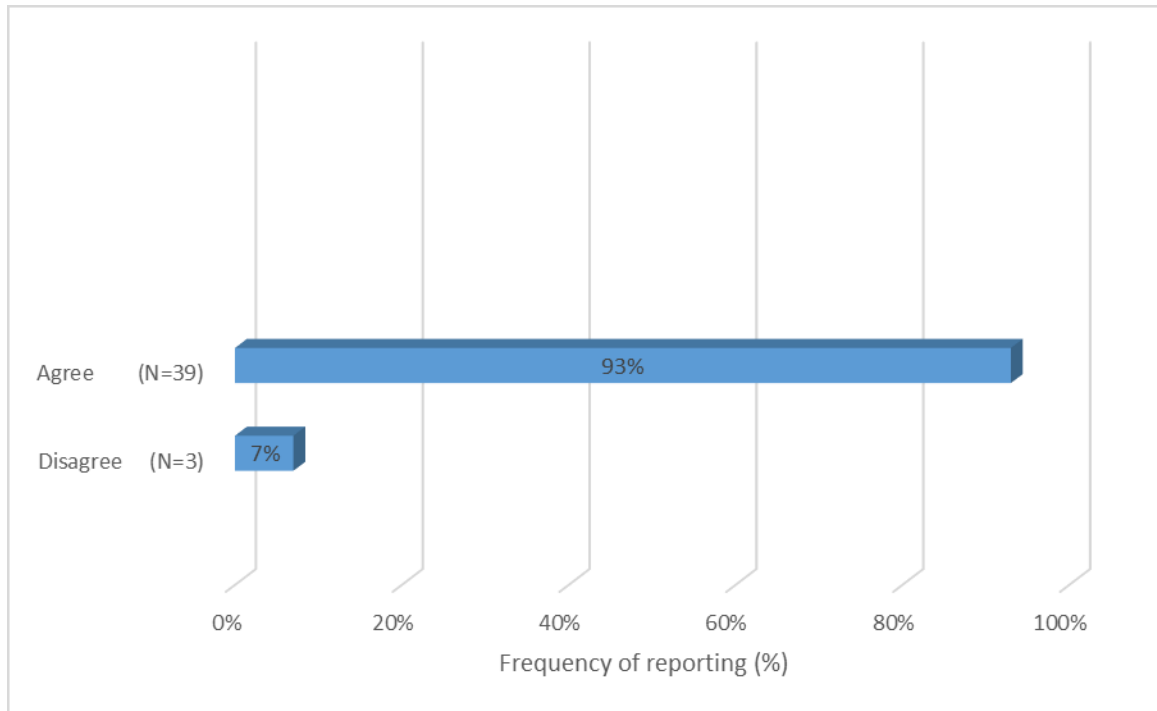
**Figure 10: Survey Response to Likelihood of Discussing Risk Factors for Diabetes**



**Table 19: Survey Response to Likelihood of Discussing Risk Factors for Diabetes.**

Answer Choices	N (%)
Very Unlikely	6(14.63)
Some Unlikely	7(17.07)
Somewhat Likely	12(29.27)
Very Likely	16(39.02)
Total	41 (100.0)

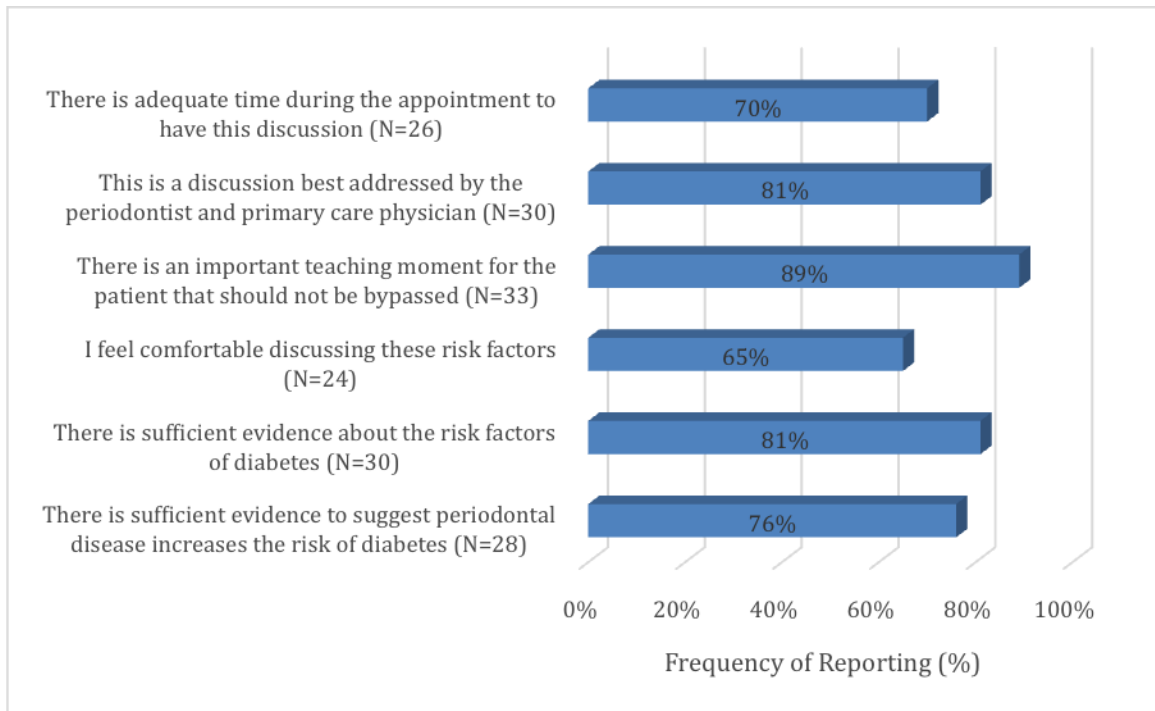
**Figure 11-Survey Response to Appropriateness of Discussing Risk Factors for Diabetes**



**Table 20-Survey Response to Appropriateness of Discussing Risk Factors of Diabetes**

Answer Choices	N (%)
Strongly Disagree	1 (2.38)
Disagree	2 (4.76)
Agree	30 (71.43)
Strongly Agree	9 (21.43)
Total	42 (100.0)

**Figure 12-Survey Response to Reasons for Discussion Risk Factors for Diabetes**



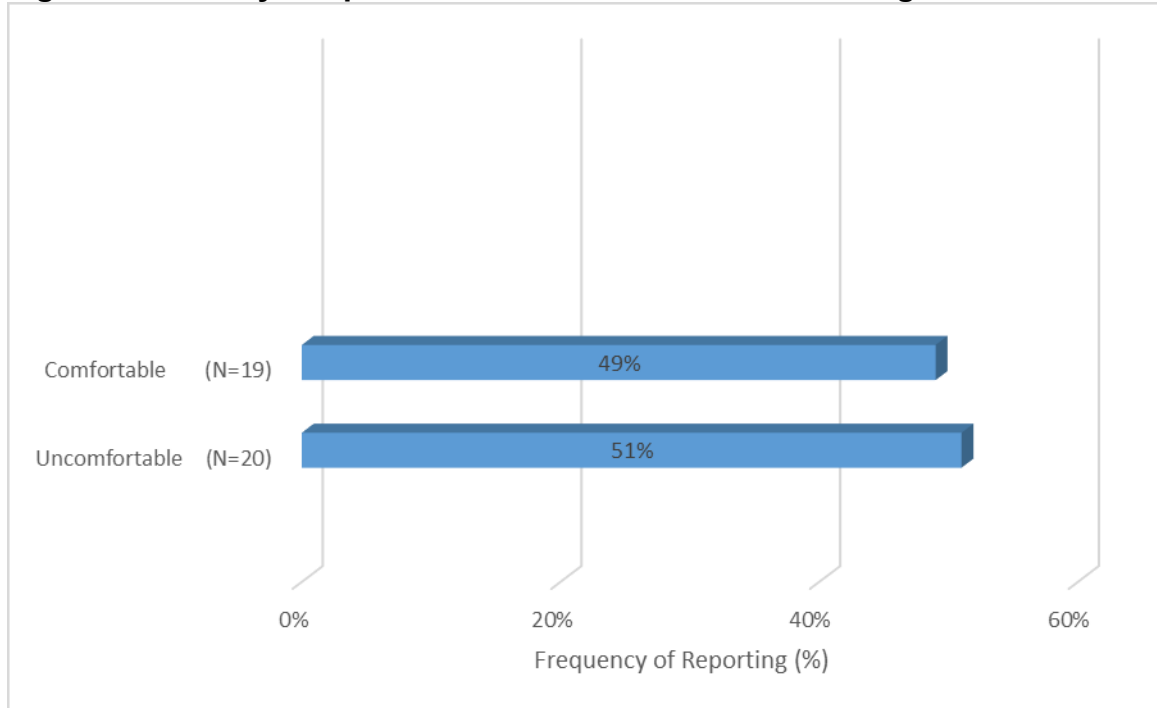
\*ranking in order of importance not summarized in this chart

**Table 21-Survey Response Rankings of Reasons for Discussion Risk Factors for Diabetes**

Answer	1*	2*	3*	4*	5*	6*	N/A	Total	Average Ranking
There is sufficient evidence of the risk factors for diabetes	21.43 % (6)	46.4 3 (13)	10.7 1 (3)	10.7 1 (3)	3.57 (1)	7.14 (2)	0 (0)	28	2.50
There is sufficient evidence to suggest periodontal disease increase the risk of diabetes	40 (12)	10 (3)	6.67 (2)	16.6 7 (5)	10 (3)	10 (3)	6.6 7 (2)	30	2.75
I feel comfortable discussing these risk factors	12.50 (3)	16.6 7 (4)	41.6 7 (10)	20.8 3 (5)	4.17 (1)	4.17 (1)	0 (0)	24	3.00
This is an important teaching moment for the patient that should not be bypassed	18.18 (6)	18.1 8 (6)	18.1 8 (6)	15.1 5 (5)	18.1 8 (6)	12.1 2 (4)	0 (0)	33	3.33
This is a discussion best addressed by both the periodontist and primary care physician	10 (3)	16.6 7 (5)	13.3 3 (4)	23.3 3 (7)	16.6 7 (5)	20 (6)	0 (0)	30	3.80
There is adequate time to have this discussion	0 (0)	3.85 (1)	15.3 8 (4)	15.3 8 (4)	26.9 2 (7)	26.9 2 (7)	11. 94 (3)	26	4.65

\* 1=most important, 2= important, 3=moderately important, 4= less important, 5=least important

**Figure 13- Survey Response for Comfort Level of Screening for Diabetes**

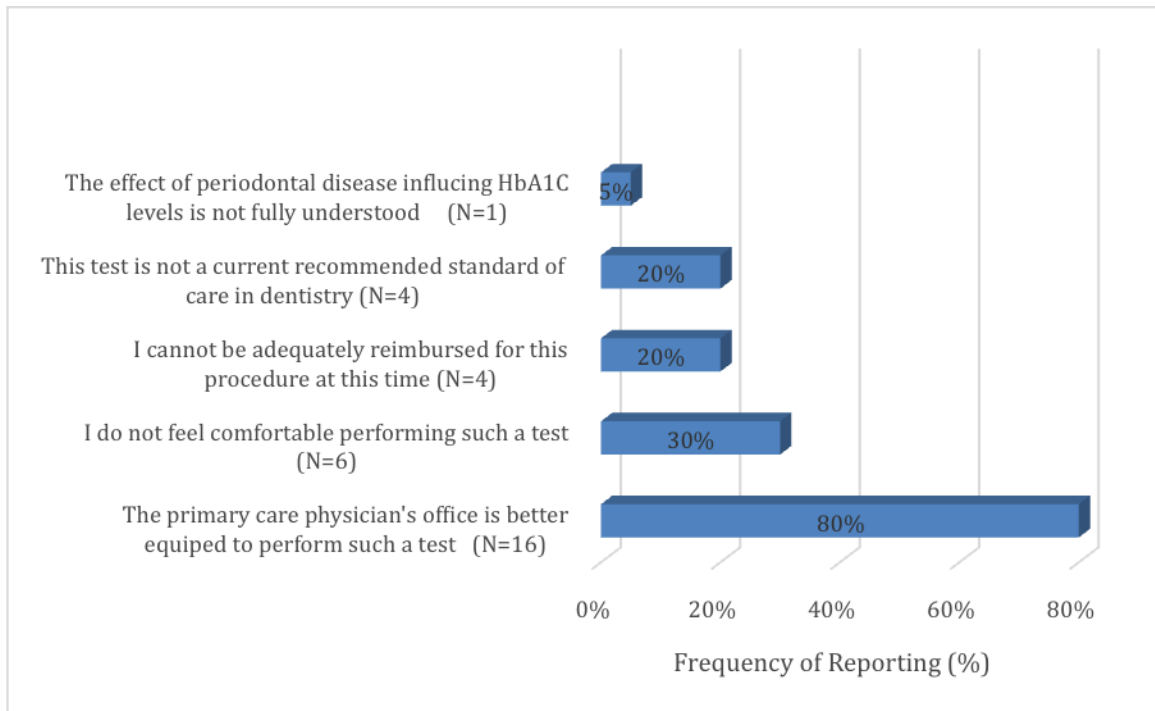


**Table 22 -Survey Response for Comfort Level of Screening for Diabetes**

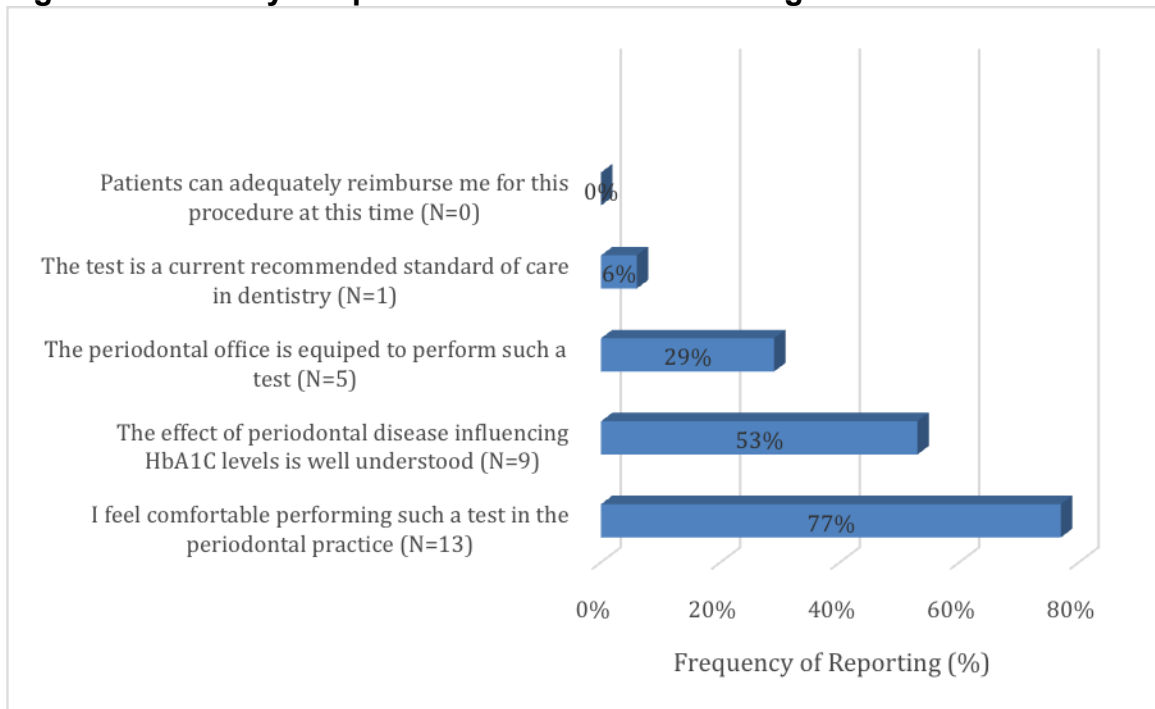
Answer Choices	N (%)
Very Uncomfortable	6 (15.38)
Uncomfortable	14 (35.9)
Comfortable	10 (25.64)
Very Comfortable	9 (23.08)
Total	39 (100.0)



**Figure 14- Survey Response for Reasons Not Screening for Diabetes**



**Figure 15-- Survey Response for Reasons Screening for Diabetes**



## Discussion

The position papers from the AAP have been discussing the two-way relationship for almost two decades. While evidence of the effect periodontal disease on glycemic control in type 2 diabetes populations has been well documented, the Joint EFP/AAP consensus report concluded that evidence is just emerging.<sup>53</sup> This EFP/AAP group also gave recommendations for future research that will strengthen what is known about this association. Approximately 76% of respondents to our survey felt that there is sufficient evidence to support periodontal disease increase the risk of type 2 diabetes, though a recent review concluded such studies are “sparse”.<sup>59</sup>

Over 50% of those comfortable in performing the test for glycemic control in the dental office felt that the effect of periodontitis on HbA1c is well understood. A review of literature identified a study (n=961) in Japan reporting over ten years, each millimeter increase in periodontal probing depth corresponded to an HbA1c of 0.13% ( $p=0.007$ ).<sup>23</sup> In contrast, a meta-analysis of 10 interventional studies of periodontal treatment found that successful periodontal therapy did not result in statistically significant changes in glycemic control in diabetic subjects, with 0.57% reduction in A1c measures ( $p=0.82$ ).<sup>24</sup> Only 456 subjects were included in all ten studies and larger studies with randomized clinical trials are needed to determine the benefit of periodontal therapy on glycemic control in patients with diabetes.

Respondents to this survey were all Members of the American Academy of Periodontology (AAP) and displayed, on average over two decades of experience in private practice. Members of this Academy have a subscription to the Journal of Periodontology and are sent position papers, consensus reports, and reviews from the AAP about topics in Periodontology. This is a professional population who we expect to be up to date in periodontal literature. Most responders were likely to discuss risk factors for diabetes (68%, n=28), and probe further about these risk factors at initial appointments for patients with a history of periodontitis. This would be expected since, medical history questionnaires, and review of the medical history by the provider, are standards of care for initial consultations. In these medical history forms, smoking, diet, and cardiovascular disease are common items that are included. (See **Appendix-Figure 18- Sample checklist for dentists provided by the AAP**) While these are risk factors for type-2 diabetes, patients positive for these risk factors may also warrant frequent oral cancer screenings, be on prescription medication, or be contra-indicated for some procedures. Thus, the 93% (n=39) responders that would probe further in patients with risk factors for diabetes is not surprising.

This study had several limitations. This study was a convenience sample of the Washington, D.C. metropolitan area. It gives an initial look at what the beliefs of local Periodontists have about the standard of care in the profession. The web-based survey made it easy to send, receive and complete the questions and no recipients requested a paper format of the survey, thus shortening the

time to receive completed surveys for analysis. Response rate may not have been increased if paper surveys were mailed. Our response rate (39%) is in the typical range from 20-47% for electronic surveys.<sup>60</sup>

The sampling the AAP members may not be representative of other practicing periodontists, who do not have easy access to the Journal of Periodontology, position papers, consensus reports, and reviews from the AAP about topics in Periodontology. This group would be expected to be the most informed group, with an information bias due to the availability of AAP publications, as their knowledge may be better than other specialists in the community. Additionally, general dentists often treat mild, to moderate forms of periodontal disease, so limiting the survey to periodontists may miss the beliefs of the standard of care in treating the many patients with early periodontal disease in this geographic area. Our findings may not be generalizable to all Periodontists and cannot be applied to all providers (general dentists) treating patients with periodontal disease.

Respondents who answer web-based e-mails may be a biased towards providers who are inter-net savvy. These individuals can access the most current literature on the web and may possess an informational bias. Additionally, a local Periodontist, with whom some of the survey recipients were acquainted, made the initial telephone calls. Thus responders may also have responded with an appeasement bias to please a fellow colleague.

Questions in the survey had closed-ended answers to which respondents were asked to choose answers. These answers were presumed to be the most likely answers based by experts in both dentistry and survey design. While such a survey is places less burden of time on respondents and is simple to analyze, it is possible however, that if the questions were open-ended, local Periodontists may have provided quite different and varied responses.

## **Conclusion**

The association of periodontitis with diabetes with type 2 diabetes is accepted by local Peridontists (using a convenience sample survey of Washington DC area Periodontists), and the attitudes and beliefs of these specialists influence the standard of care in treating dental patients. When asked if it was appropriate to probe further about of diabetes risk factors in patients with periodontal disease and no diabetes diagnosis, most respondents (92.9%) agreed (agreed/ strongly agreed, n=39).

The local Periodontists surveyed felt, in order of most important to least important: there is sufficient evidence about the risk factors for diabetes, there is sufficient evidence to suggest that periodontal disease increases the risk of diabetes, they were comfortable discussing these risk factors, this is an important teaching moment for the patient that should not be bypassed, this is a discussion best addressed by both the periodontist and the primary care physician, and

there is adequate time during the appointment to have this discussion. This survey suggests that practicing periodontists are aware that there is an association between periodontal disease and the onset of type 2 diabetes.

Those surveyed also appear aware of the importance of HbA1c testing in assessing glycemic control, whether this test is performed in the dental office or medical setting. This appears to parallel the consensus report of the Joint European Federation/ American Academy Workshop (EFP/AAP) guidelines to dentists for patients without a diabetes diagnosis, but obvious risk factors for type 2 diabetes, where patients:

“ should be informed of their risk for having diabetes, assessed using a chair-side HbA1C test, and/or referred to a physician for appropriate testing and diagnostic care.”<sup>53</sup>

Overall, the local Periodontists responding to the survey appear to be implementing current and best practices recommended as the standard of care in dentistry.

## CHAPTER 5

### Conclusions

#### *Summary*

Periodontal disease has been proposed as one source of inflammation that might predispose adults to developing diabetes. Though the hypothesis of a bidirectional pathway between periodontal disease and diabetes has been proposed, few studies have addressed periodontal disease before the occurrence of diabetes.<sup>19</sup> Localized periodontal inflammation is now known to have systemic effects on general health.<sup>40,41</sup> Compromised oral health may increase the risk of a pre-diabetic status mediated through inflammation. Our study, which used both clinical exams and markers for inflammation, looked at the association of periodontal disease exposure and its' association with pre-diabetes and diabetes risks.

In our cross- sectional analysis, we found clinical periodontal measures for inflammation were associated with the likelihood of impaired fasting glucose. This association seemed to hold true for individuals with only slight bleeding upon probing, with deeper probing measurements, or more severe gingival bleeding upon probing. However, a dose response relationship was not observed with increasing severity of periodontal inflammation. Compared with individuals with normo-glycemic levels, adults with pre-diabetes using fasting glucose levels, had



an increased odds of periodontal clinical inflammation that remained even after adjustment for lifestyle and co-morbidity covariates. Participants with more severe periodontal clinical inflammation had an increased odds of impaired fasting glucose. Compared to individuals in Category I, the odds ratio for impaired fasting glucose in Category V was 2.1 (95% CI: 1.6-2.8) in an unadjusted model. This relationship remained in the fully adjusted model with an odds ratio of 1.5 (1.1--2.1) in this highest category (one or more sites with a probing depth >4 mm and bleeding upon probing  $\geq 50\%$ ).

To further explore the relationship, between prediabetes and periodontal inflammation, we performed four additional analyses using markers of systemic inflammation (serum antibody levels to the periodontal pathogens *Porphyromonas gingivalis* and *Actinobacillus actinmycetemcomitans*), and markers of localized inflammation (gingival crevicular fluid levels of IL-1 $\beta$  (GCF-IL-1 $\beta$ ) and gingival crevicular fluid levels of prostaglandin (PG-E2) However, no significant association was observed between pre-diabetes or undiagnosed diabetes and any of those inflammation markers.

In a longitudinal design, adults with clinical periodontal measures for inflammation were associated with incident diabetes. During 13.84 years of follow up 1,967 individuals developed Type 2 diabetes of the total (n= 5,819) participants initial visit 4. Compared with individuals with minimal bleeding and probing measures, the hazard of incident diabetes appears to be 1.2 times higher in adults with moderate to severe clinical periodontal inflammation (Category IV

and Category V both having 95%CI: 1.0-1.6,  $P < 0.001$ ). However Category III did not display this increasing significant trend with a hazard ratio of 1.0 (95% CI: 0.8-1.20). Our results did not support the findings of CVD outcomes where systemic markers for periodontal inflammation were associated with an increased risk of cardiovascular disease. The hazard of incident diabetes appeared to be the same in adults with high levels vs. low levels of antibodies to *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*. Similarly, no increase in incident diabetes could be seen in high vs. low levels of gingival crevicular fluid levels of IL-1 $\beta$  and PG-E2.

Using a convenience sample survey of Washington DC area Periodontists, and the attitudes, beliefs, and the standard of care in treating dental patients at risk for type 2 diabetes were assessed. Respondents to this survey were all Members of the American Academy of Periodontology (AAP) and displayed, on average over two decades of experience in private practice. Approximately 76% of respondents to our survey felt that there is sufficient evidence to suggest that periodontal disease increases the risk of type 2 diabetes, though a recent review concluded such studies are “sparse”.<sup>59</sup> Almost 50% of those surveyed were comfortable in performing the HbA1c test for glycemic control in the dental office and of those comfortable, 53% felt that the effect of periodontitis on HbA1c is well understood.

## ***Overall Discussion***

Our studies supports that hypothesis that clinical periodontal inflammation is associated with impaired glucose tolerance and this exposure may increase the risk of incident diabetes several years later. An increase in bleeding upon probing appears to be both cross-sectionally associated with impaired fasting glucose and longitudinally associated with the onset of incident diabetes. A dose-response relationship was not observed with increasing severity of periodontal inflammation. These results are to be expected since probing depths give a measure of prior periodontal attachment loss, while bleeding upon probing assesses current inflammation. A periodontal exam that combines probing depth and bleeding scores gives an accurate assessment of prior and current periodontal status, and both are used together in the examination and diagnosis of dental patients in the clinical setting.

An assessment of risk of impaired fasting glucose may be performed easily in the dental office. This requires a dental exam commonly and currently performed by dental providers. The use of immunoassays, which are costly, and technically demanding, may not be necessary to define the relationship of periodontal disease with pre-diabetes.

Presumably, the prevention of gingival inflammation may reduce the risk of diabetes onset. The reduction of periodontal disease, and then diabetes, has potential to slow the growing epidemic proportions of both diseases. Our studies contribute to the sparse evidence for the association of periodontal association with pre-diabetes and future incident diabetes. Additionally, we have provided preliminary evidence to support the hypothesis that exposure to even mild gingival inflammation may have systemic effects on glycemic control. This finding is novel, since other studies found a dose-response relationship, with more severe inflammation having more effect on glycemic control and type 2 diabetes.<sup>22, 23</sup>

The Atherosclerosis Risk in Communities (ARIC) Study, a community-based prospective cohort provided a rich database with which to assess the effect of periodontal disease exposure on incident diabetes. This study helps answer the recent call by the Joint EFP/AAP consensus report for studies with comprehensive clinical data, extent and severity of periodontal disease, level of glycemic control, and consideration of local and systemic pathways affected by both periodontal disease and diabetes.<sup>53</sup> Our studies served to contribute to the body of evidence that is largely lacking in the directionality of periodontal disease and subsequent incident diabetes.

## ***Implications***

The strength of this overall dissertation is that it serves to contribute to the lack of evidence to support the directionality of periodontal disease exposure and the associated risk of pre-diabetes and diabetes. The recent recommendation for future research in this area by the for more evidence in consensus report of the Joint European Federation/ American Academy Workshop (EFP/AAP) includes a “comprehensive assessment of clinical measures of periodontal inflammation and biochemical markers of inflammation in blood and saliva”.<sup>53</sup> Our studies used a database with complete periodontal assessments, serum antibody levels to periodontal pathogens, and gingival crevicular fluid levels of inflammatory markers to assess periodontal disease exposure.

The use serum antibody levels to periodontal pathogens as a surrogate for disease exposure is controversial. While validated in other studies<sup>51,54</sup>, use of antibody levels to *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* as measure of past severity of periodontal inflammation is not accepted as a standard measurement of periodontal disease by the Academy of Periodontology and not a current recommendation for assessment of type 2 diabetes risk. However, these markers have been used in mechanistic studies of cardiovascular disease risk, and helped suggest in our study that a different biologic pathway may be involved when assessing type 2 diabetes risk.

This data will help define the biologic mechanisms and provide direction for future interventions.

As the prevalence of diabetes and periodontitis increases, dentists will likely see an increasing number of patients with diagnosed and undiagnosed diabetes. The American Dental Association now recommends that dental offices be equipped with glucometers. Patient education about the importance of prevention and treatment of periodontal disease and diabetes is the role of both physicians and dentists.<sup>61</sup>

Insurance coverage for dental care should be mandated for people with diabetes. The health of the public will be served by public policies which focus on the prevention and control of periodontitis and diabetes.<sup>62</sup> Since the prognosis of periodontal disease is best treated at its earliest stages, programs aimed at patient education and health promotion may limit the burden of sequelae associated with diabetes and periodontal disease.

### ***Future Research***

Since even minimal bleeding upon probing in this study was associated with prevalent pre-diabetes and future incident diabetes, a strong case is made to support patient education for prevention of periodontal disease to and study

the effect of prevention of even mild periodontal inflammation on impaired glucose tolerance and diabetes.

Assessment younger populations with mild gingival inflammation, followed longitudinally, may provide evidence of the earliest effects of periodontal inflammation on the risk of impaired glucose levels and incident diabetes. Studying gingivitis, the earliest form of periodontal inflammation, may yield the most sensitive ascertainment of the effect of subtle changes of oral health on diabetes.

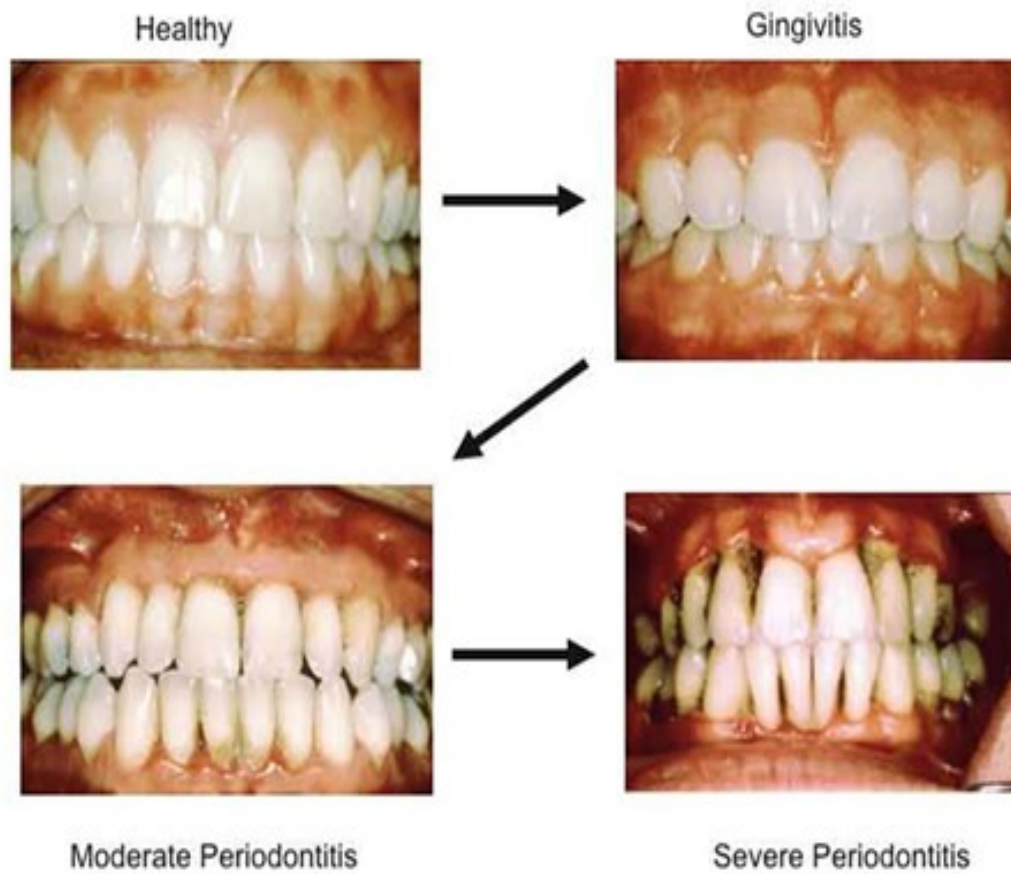
The cost-effectiveness and outcomes of screening programs in the pre-diabetic state should be reliably assessed in relation to oral health. This can be performed if medical providers routinely refer “at risk” individuals to dental professionals.

Finally, the relationship between periodontal disease and diabetes is complex and not likely to be understood by a single study regardless of its design. Synthesis of existing and future studies will be helpful in elucidating these relationships and provide the direction for public health policies aimed at reducing their burden on the general public.

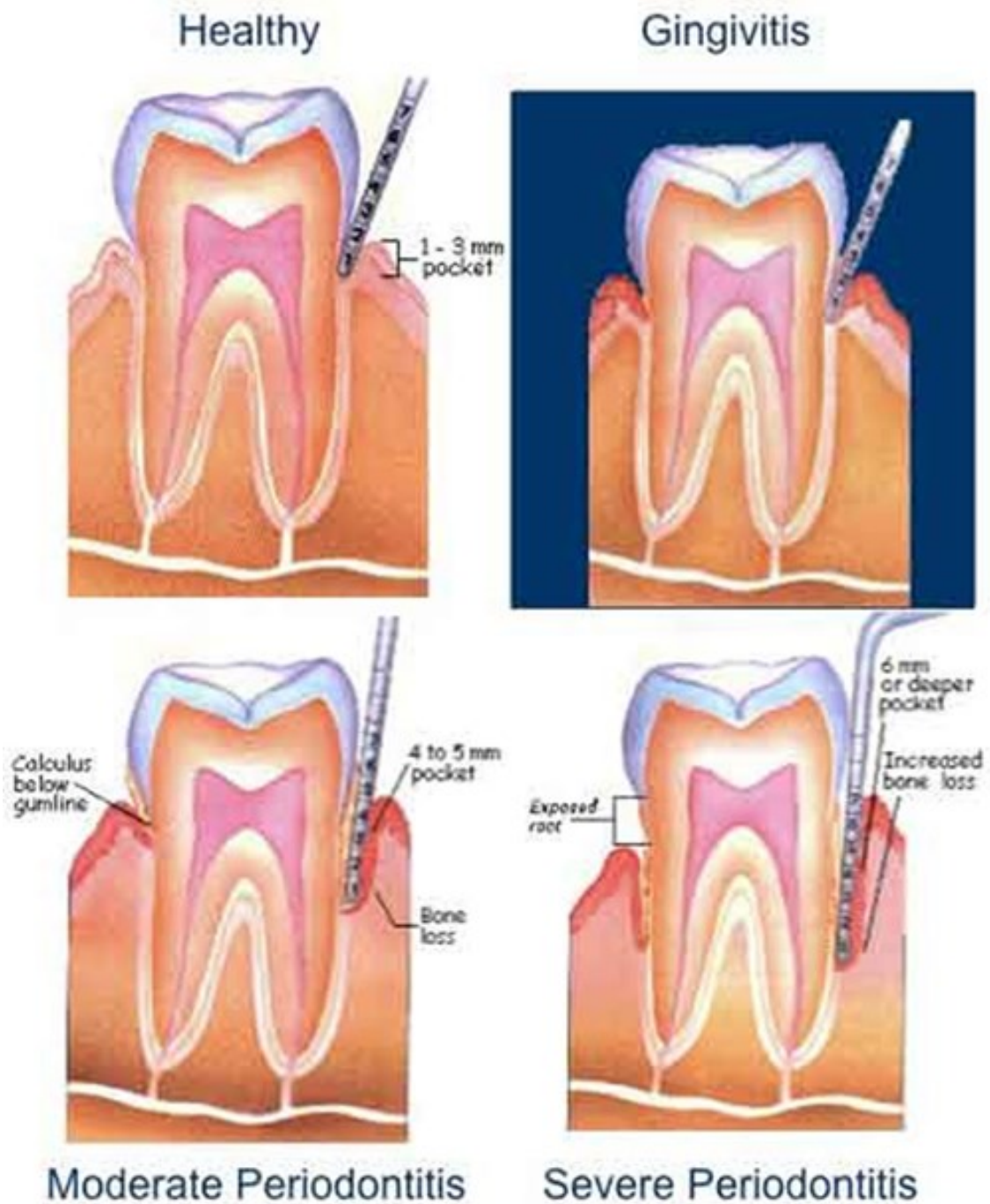
## **Appendix**



**Figure 16: Clinical Appearance of Stages of Periodontal Disease**



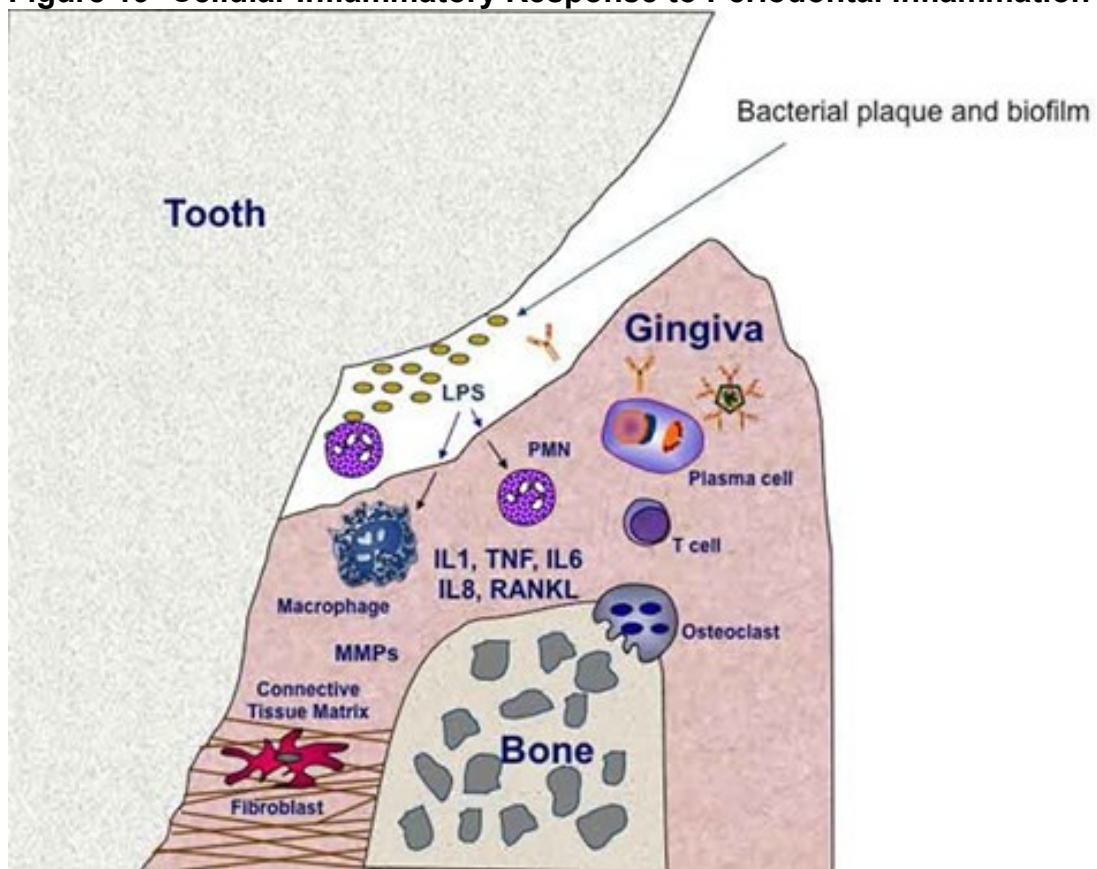
**Figure 17: Probing Measurements By Severity of Periodontal Inflammation**



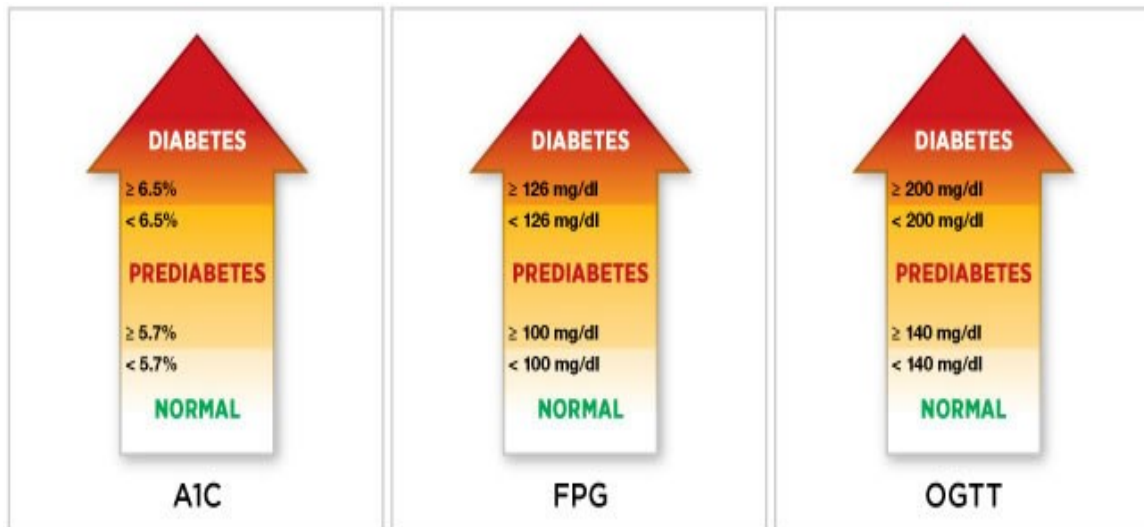
**Figure 18: Example of a Checklist for Periodontal Patients in the Clinical Setting** <sup>63</sup>

COMPREHENSIVE PERIODONTAL EVALUATION CHECKLIST		
Patient Name: _____		
Clinician: _____		
Date of Evaluation: ____ / ____ / ____		
<b>Instructions:</b> - Review each of the six elements listed below - Mark your initial by each "Specific Consideration" - Refer to other patient information, radiographs etc. in the "Notes" section		
<b>1. TEETH, DENTAL IMPLANTS AND SUBGINGIVAL AREA</b>		
<b>Initials</b>	<b>Specific Considerations</b>	<b>Notes</b>
	pocket depths	
	width of keratinized tissue	
	gingival recession	
	attachment level	
	bleeding on probing	
	furcation status	
	presence of inflammation	
<b>2. PLAQUE/BIOFILM</b>		
<b>Initials</b>	<b>Specific Considerations</b>	<b>Notes</b>
	presence, degree, and/or distribution of plaque/biofilm	
	presence, degree, and/or distribution of calculus	
<b>3. DENTITION</b>		
<b>Initials</b>	<b>Specific Considerations</b>	<b>Notes</b>
	caries	
	proximal contact relationships	
	endodontic/periodontal lesions	
	status of dental restorations and prosthetic appliances	
	other tooth or implant related problems	
<b>4. OCCLUSION</b>		
<b>Initials</b>	<b>Specific Considerations (but not be limited to)</b>	<b>Notes</b>
	degree of mobility of teeth and dental implants	
	occlusal patterns	
	fremitus	
<b>5. DIAGNOSTIC QUALITY RADIOGRAPHS</b>		
<b>Initials</b>	<b>Specific Considerations</b>	<b>Notes</b>
	quality/quantity of bone	
	bone loss patterns	
<b>6. DISCUSSION OF PATIENT RISK FACTORS</b>		
<b>Initials</b>	<b>Specific Considerations</b>	<b>Notes</b>
	age	
	diabetes	
	smoking	
	cardiovascular disease	
	other	

**Figure 19- Cellular Inflammatory Response to Periodontal Inflammation**



**Figure 20: Diagnostic Criteria of Pre-diabetes and Diabetes by Glycemic Test<sup>64</sup>**



## **Preamble to Telephone and Internet Survey from Chapter 4**

### **Endorsement by the Dean of Howard University College of Dentistry:**

Dear Colleagues,

Periodontal disease is the most common inflammatory condition worldwide and diabetes is quickly becoming a global epidemic. The bidirectional pathway of periodontal disease and diabetes is not fully understood. While consistent evidence has shown that diabetes is related to periodontitis, emerging evidence suggests that periodontal disease may increase the risk of diabetes onset.

Your participation in the survey will help answer important questions regarding the direction of future research and patient education in this important area of oral health. In addition we will better understand what is the attitude of current best practices in managing periodontal patients at risk for diabetes.

I hope that you will take the time to complete these six questions. We expect that this survey will take 3-5 minutes to complete. Your consent to participate is assumed by your completing the survey. All data will remain confidential and will only be published in aggregated form. Individual respondents will not be identified in publications.

Your expertise in this field is greatly appreciated. Many thanks for your help.

Sincerely,

Leo (Signed by Dean Leo Rouse) [Reviewed by Dean Rouse on 11/14/13, electronic signature was added to web-based survey]

Leo E. Rouse DDS, FACD  
Past President of American Dental Education Association  
Interim Deputy Provost for Health Sciences  
Dean of Howard University College of Dentistry

#### Survey Questions from Chapter 4:

1) If your patient has a history of periodontal disease, but no reported medical history of diabetes, how likely are you to discuss the risk factors of diabetes (such family history of diabetes, smoking, diet, exercise, cardiovascular disease) during the initial consultation appointment:

Very Unlikely      Somewhat Unlikely      Somewhat likely      Very Likely

2) Do you think it is appropriate to probe further about risk factors of diabetes (such family history of diabetes, smoking, diet, exercise, cardiovascular disease) in your patients without a current medical history of diabetes?

Strongly Disagree      Disagree      Agree      Strongly Agree

3a) You answered that you do not agree/strongly disagree that asking about risk factors of diabetes is appropriate in your non-diabetic patients. Is this because: (Number all that apply, if any, in order of importance, i.e. #1-most important reason)

- i) There is not enough evidence to suggest periodontal disease increases the risk of diabetes.
- ii) There is not enough evidence about the risk factors for diabetes.
- iii) I don't feel comfortable discussing these risk factors.
- iv) There is insufficient time during the appointment to have this discussion.
- v) This is a discussion best addressed by the patient's primary care physician.
- vi) I think the patient will not expect the Periodontist to do this.

3b) You answered that you agree/strongly agree that asking about risk factors of diabetes is appropriate in your non-diabetic patients. Is this because: (Number all that apply, if any, in order of importance, i.e. #1-most important reason)

- i) There is sufficient evidence to suggest periodontal disease increases the risk of diabetes.
- ii) There is sufficient evidence about the risk factors for diabetes.
- iii) I feel comfortable discussing these risk factors.
- iv) There is adequate time during the appointment to have this discussion.
- v) This is a discussion best addressed by both the periodontist and the primary care physician.

- vi) This is an important teaching moment for the patient that should not be bypassed.

4) In patients that do not have a diagnosis of diabetes, but have obvious risk factors for diabetes, how comfortable are you in assessing glycemic control using a chair-side HbA1C test?

Very Uncomfortable   Uncomfortable                      Comfortable   Very Comfortable

5a) You answered that you were not comfortable in performing a chair-side HbA1C test. Is this because (Check all that best apply):

- i) The effect of periodontal disease influencing HbA1C levels is not fully understood.
- ii) The primary care physician's office is better equipped to perform such a test.
- iii) I do not feel comfortable performing such a test in the periodontal practice.
- iv) I cannot be adequately reimbursed by dental insurance companies for this procedure at this time.
- v) This test is not a current recommended standard of care in dentistry.

5b) You answered that you were comfortable in performing a chair-side HbA1C test. Is this because (Check all that best apply):

- i) The effect of periodontal disease influencing HbA1C levels is well understood.
- ii) The periodontal office is equipped to perform such a test.
- iii) I feel comfortable performing such a test in the periodontal practice.
- iv) Patients for this procedure can adequately reimburse me at this time.
- v) This test is a current recommended standard of care in dentistry.

The last question tells us something about you as a survey respondent:

6) I have been practicing my periodontal specialty for approximately \_\_\_\_\_ years.



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**Curriculum Vitae**  
**Indra Zena Mustapha**

Place of Birth- Edmonton Alberta, CANADA  
Date of Birth- November 3, 1970  
701 Pennsylvania Ave NW #1028  
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**A. Positions and Honors**

**Positions and Employment:**

1995-1998	Periodontal Residency, Mayo Clinic
1998-2006	Private practice in Periodontics, Washington, D.C.
1999-2005	Assistant Professor, Howard University College of Dentistry
2000-2005	Assistant Professor, University of Maryland College of Dentistry
2006- present	Private practice in Periodontics, Washington, D.C.
2011-present	Assistant Professor, Howard University College of Dentistry

**Other Experience and Professional Memberships**

1995-99	Member of American Academy of Periodontology (AAP)
1997	Participant AAP Annual Meeting, New Orleans, LA
1998	Presenter, IADR "The effect of P-15 Growth Factor Around Endosseous Implants"
1999-present	Diplomate of American Board of Periodontology
2002	Participant AAP Annual Meeting, New Orleans, LA
2007	Ad hoc reviewer, Indian Journal of Dental Research
2007	Participant AAP Annual Meeting, Washington, DC
2006-2014	Graduate Training Program in Clinical Investigation, Johns Hopkins University

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### **Honors/Awards**

1995	Dean's Award Howard University College of Dentistry
1995	Who's Who Recognition American Colleges
2006	Teacher of the Year in Clinic Modules- Howard University
2006-2011	T-32 NIH Training Grant at NYU and Johns Hopkins

### **B. Peer-reviewed Publications (in chronological order)**

Mustapha, IZ, Reeve, CM, Sheridan PJ. The effect of P-15 growth factor around endosseous implants. (Abstract) IADR, 1998.

Meraw, S, Mustapha, IZ, Rogers, R. The effect of tobacco on oral mucosa. Dental Clinics of North America. 1998.

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